

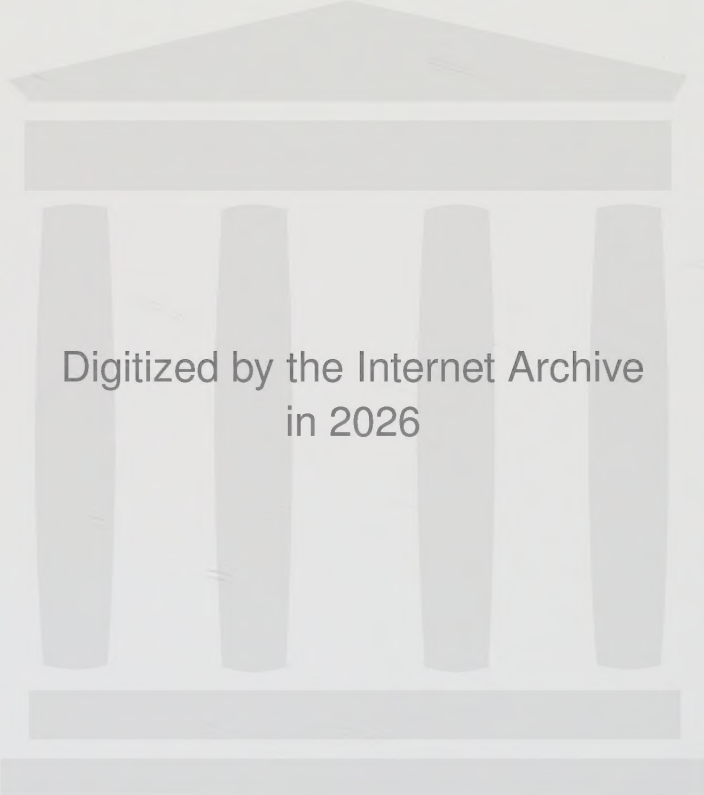
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HYPERSPLENISM

A clinical and experimental study



Anders Alwmark



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HYPERSPLENISM
A clinical and
experimental study

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leg. läk., Klm.

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Title and subtitle HYPERSPLENISM. A clinical and experimental study.			
Abstract The clinical part of this study showed that repeated partial embolization of the spleen in patients with hypersplenism secondary to portal hypertension improved the thrombocytopenia and diminished the number of bleeding episodes. The mortality was about half of the mortality reported by others after splenectomies in cirrhotic individuals. The discomfort to the patients, e.g. pain, fever and pleural affection was mostly short-lasting and easily treated. The long-term follow-up demonstrated the need for further embolizations after 2-3 years in order to preserve the "normosplenic" state, because of the regenerative capacity of the splenic remnant. The experimental studies were all performed on rats made hypersplenic by intraperitoneal injections of methylcellulose. The large spleen was reduced and the pancytopenia was normalized by partial splenectomy or ligation of the vessels supplying part of the spleen. One third reduction normalized the blood picture whereas two thirds reduction increased the risk for infectious complications. The animal model was also used to study the susceptibility of hypersplenic rats to pneumococcal challenge. Using standardized, frozen inocula of <i>Streptococcus pneumoniae</i> the hypersplenic rats were found to be significantly more susceptible to such challenge than normal rats. Hypersplenic rats made asplenic by ligation of the splenic artery were found to develop abscesses in the infarction tissue. Pneumococci to these animals caused a significantly higher mortality than that found in hypersplenic rats given the same dose of pneumococci. Hemostasis parameters were also studied in "hypersplenic" rats. The decrease in platelet count described above was accompanied by impaired aggregation ability of the thrombocytes. Bleeding tendency and operative blood loss during a standardized liver resection increased in hypersplenism. Splenectomy normalized the bleeding tendency although improvement of the platelet aggregation was not noticed.			
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Date 1982-04-14

From the Department of Surgery, University of Lund

S-221 85 Lund, Sweden

HYPERSPLENISM

A clinical and experimental study



by

Anders Alwmark



Lund 1982



Maria 8år

... given standard food pellets and water ad libitum.

This thesis is based on the following papers:

- I Evaluation of splenic embolization in patients with portal hypertension and hypersplenism.
Alwmark A, Bengmark S, Gullstrand P, Joelsson B, Lunderquist A, & Owman T. Ann Surg, in press.
- II Treatment of hypersplenism - an experimental study in the rat.
Alwmark A, Bengmark S, Börjesson B, & Gullstrand P. Eur Surg Res, in press.
- III Increased susceptibility of hypersplenic rats to infection with pneumococci.
Alwmark A, Christensen P, Gullstrand P, & Schalén C. Acta Path Microbiol Immunol Scand. Sect B, in press.
- IV Increased susceptibility to pneumococci after ligation of the splenic artery in experimental hypersplenism.
Alwmark A, Bengmark S, Christensen P, Gullstrand P & Schalén C. Submitted for publication.
- V Hypersplenism - effect on hemostasis. An experimental study in the rat. Alwmark A, Bengmark S, Gullstrand P, & Zoucas E. Submitted for publication.

In the text the above articles are referred to by their Roman numerals.

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ABBREVIATIONS

ADP	adenosine-diphosphate
APT-time	activated partial tromboplastine time
CFU	colony forming unit
OHSI	overwhelming hypersplenic infection
CML	chronic meylotic leukemia
HD	Hodgkin's disease
OHSI	overwhelming hypersplenic infection
OPSI	overwhelming postsplenectomy infection
PTP	percutaneous transhepatic portography
RES	reticuloendothelial system
SST	selective symptomatic treatment
WBC	white blood cell

INTRODUCTION

Historical notes

For more than 2 000 years the spleen was an organ shrouded in mystery, most often connected with negative experiences such as depression, anger and pain, but also with merry humour and speed in running. Aristotle found it an important organ but not essential, as congenitally asplenic persons could pursue a fairly normal life (1). Erasistratus stresses this concept and claimed that "nature had shaped nothing unnecessary but the spleen". Some 400 years later the concept of the four temperaments and their humoral representation was constructed by Galen. The spleen was supposed to cleanse the blood from the black bile, which in excess made the person sad and melancholic. The allegory has been used by many, for example, the Swedish 19th century poet, Tegnér, who described his sadness when he had to leave the university town of Lund as a splenetic black elf gnawing at his heart, and Burnett who composed "My Melancholy Baby" for the muted saxophone.

A more scientific approach to the spleen was made by the middle-aged anatomist Malpighi who in 1669 described some of its microanatomic features including the Malpighian corpuscles (2). He introduced the modern view of the spleen as being an organ belonging to the vascular system and located in the splanchnic bed (2).

One of the first "clinical" observations on patients with an enlarged spleen was presented in 250 AD by Samonicus who found that people with a swollen spleen always had a stupid laugh which disappeared when the spleen was removed. Such splenectomized men became more agile and active according to Winston in his Anatomy Lecture (1659). Even much later writers were fascinated by the spleen. In India the large spleen was compared with the back of a turtle, leading to the suggestion of the following therapy: hot needles plunged into the organs, beating the left hypochondrium or drinking the water from a blacksmith's shop. (For historical references, see Ask-Upmark (3).

The physiologic role of the spleen with its relation to the blood as it is known today was not discussed until the middle of the 19th century. The German pathologist Virchow described in 1846 a patient with a combination of "white blood" ("Weisses Blut") and large spleen, a condition which he called "splenomyelogenous leukemia" (4). Another German, Gretscl, was the first to use the term "splenic anemia" (5), which Banti later on described extensively in a series of publications beginning in 1882. Banti concluded that a "noxa" originated from the enlarged spleen and caused anemia, leukopenia and liver cirrhosis, a combination since then known as "Banti's disease" (6). Banti is thus given the credit for observing the relationship between the spleen, the liver and the bloodforming apparatus. At the beginning of this century further cases of splenomegaly in association with anemia, leukopenia and now even thrombocytopenia were

described by Eppinger (7) as well as Frank (8) in Berlin and by Chauffard in Paris. The last-mentioned writer was the first to use the term "hypersplenism" (9). This was in 1907, i.e. 75 years ago.

The evidence against the spleen as a noxious organ in some blood diseases grew stronger and planned splenectomies in so called blood dyscrasias as well as in liver cirrhosis with splenomegaly were performed with increasing frequency. Despite the words of warning by Morris and Bullock in 1919 (10), that splenectomized patients were more susceptible to infection, these operations grew in popularity. This was the trend for more than fifty years. It is only recently that the increasing number of reports on overwhelming postsplenectomy infection (OPSI) (10-14) has made surgeons more reluctant to splenectomy in children as well as in adults (11-15).

Anatomy of the spleen

The spleen is of mesenchymal origin derived from the dorsal mesogastrium and is one of the first lymphoid organs to appear in embryonic life. The normal adult spleen weighs about 150 g (1, 16, 17) with a range of 50-250 g much depending on the blood content. It contains the largest amount of lymphoid tissue and is the largest unit of the reticuloendothelial system in the human body. Thus "the spleen is a big lymph node placed in the portal circulation system without any proper lymphatic supply" (18).

Soon after entering the hilus the splenic artery branches into the trabecular arteries and then into the central arteries of the white pulp, here surrounded by the white lymphoid tissue of the Malpighian body. From here on, the microcirculation of the spleen is still a matter for debate. The question for more than 200 years has been whether the circulation through the red pulp is open or closed. Malpighi presented the closed circulation theory with the arterioles in the red pulp directly followed by small venules (2) whereas Billroth, 200 years later, gave arguments for the open circulation with the arterioles terminating freely into large, thin-walled sacs drained into the red pulp veins, the trabecular veins and the main splenic vein (19). A third theory was proposed by Knisely in 1936 who described the "divided circulation" with some blood passing the direct way of Malpighi but the greater part entering the fairly tight sinuses with a possibility to store cells if the supposed afferent and efferent sphincters closed synchronously (20). The modern view obtained by light and electron microscopic studies largely supports the theory of Knisely with the exception that the splenic sinuses are not tight but lined by an endothelium with pores through which the blood cells can enter the intervening cords (21,22). This concept was recently confirmed by injecting plastic microspheres of a specific size which showed that 90 % of the blood passed via the open route and 10 % via the "closed route" (23). Phagocytic cells form a network in the splenic cords, which together with the endothelial cells of the sinuses also belong to the reticuloendothelial system, giving an intimate contact between the RES and the blood.

Splenic function and hypofunction

The spleen acts as a filter in the portal circulation receiving about 5 % of the blood volume per minute (24). The unique circulation makes the spleen suitable for clearance of blood corpuscles and particulate antigen (25). This capacity is of great importance when poorly opsonized bacteria have to be removed (26). The spleen also elaborates a specific immune response especially against particulate antigen starting with the IgM-antibody response (27). A "non-specific" immune response is achieved via the synthesis of the opsonizing proteins, i.e. tuftsin and properdin. Tuftsin is a tetrapeptide that coats leukocytes to promote phagocytosis (28,29) and properdin is a component of the alternative pathway of complement activation (30). Splenectomized or hyposplenic patients frequently show an impaired opsonic activity as well as decreased levels of IgM-antibodies (31). Such patients run an increased risk of acquiring serious infections especially from encapsulated bacteria such as pneumococci, staphylococci and Hemophilus influenzae. The greater susceptibility to infection was first noticed in splenectomized children (11,13) but during recent years several reports of OPSI in adults have also been published (14,15). The highest frequency of infections was encountered in hyposplenic patients with an additional immune-defect such as sickle cell anemia or malignancies e.g. Hodgkin's disease (HD) (11). However, even normal adults splenectomized because of abdominal trauma exhibit as much as an 80-fold increased risk of fatal postsplenectomy sepsis when compared with non-splenectomized adults (11, 32).

Splenic hyperfunction

The works by Doan and Dameshek in the late forties established the term "hypersplenism" (33,34). The syndrome consists of a combination of a) splenomegaly; b) anemia and/or leukocytopenia and/or thrombocytopenia; and c) compensatory hyperplasia of the bone marrow. Furthermore, the syndrome is characterized by improvement after splenectomy. In a few cases the "hyperactive" spleen is not much enlarged (idiopathic thrombocytopenic purpura) whereas in other cases the spleen by its size causes mechanical problems for the patient, who has no noticeable changes in the peripheral blood. Thus, Eichner redefined hypersplenism as a condition where "the spleen in question has become more harmful than beneficial" (24). With the rare exceptions mentioned above the basis of hypersplenism is almost always the splenomegaly which could be secondary to:

1. work hypertrophy from cell destruction: hereditary spherocytosis, thalassemia major, sickle cell anemia (in the beginning of the disease).
2. work hypertrophy from immune response - infections (e.g. endocarditis, mononucleosis, malaria), Felty's syndrome, chronic renal insufficiency.

3. congestion - liver cirrhosis, portal vein or splenic vein thrombosis
4. myeloproliferation - myelofibrosis
5. infiltration - lipoidoses, sarcoidosis, amyloidosis
6. neoplasm - lymphoma, leukemia, metastatic cancer.

The result of the splenomegaly, whatever its cause, is almost always: shortage of one or more of the peripheral blood elements. The mechanism by which this reduction occurs has been much debated, and at least two different modes of action have been proposed:

1. RES-hyperplasia causing increased sequestration and phagocytosis of blood elements (33,35,36,37).
2. A humoral factor produced by the spleen depressing the bone marrow (34,38,39).

A third alternative was introduced in 1967 by Jandl and Aster who considered splenic pooling of the blood elements to be the important mechanism (40).

The normal erythrocytes seem never to be destroyed in the spleen although its ability of sequestration and destruction of abnormal or injured red cells is pronounced. In the hypersplenic spleen there is still no sequestration of normal cells but a slower transit of the cells through the spleen is detectable (40). The red cells hereby retained may represent a pool of up to 20 % of the total red cell mass. It is occasionally possible to demonstrate a higher turn-over of red cells in pronounced hypersplenism but this phenomenon is explained by the lack of glucose in the packed sinusoids (41). Thus, mature red cells depending on continuous glycolysis (in contrast to the reticulocytes) are injured. Other authors, however, have questioned this explanation reporting a pronounced anemia combined with a marked decrease of red cell survival in patients as well as in animals with an experimentally induced hypersplenism (36,37).

Radioactively labelled thrombocytes are found to be pooled in the spleen to an important extent in the normal and even more in the hyperfunctioning spleen (25-50 % of the total platelet count in hypersplenism) (40,42). Their small size seems to allow the platelets free passage to and through the sinusis without trapping or metabolic injury. It is possible to mobilize the splenic pool of trombocytes by injection of adrenalin (42). Experiments have also shown a free communication between circulating and pooled platelets, and a feedback system to the bone marrow has been suggested. Thus, imbalance in the equilibrium between the blood marrow and the circulating trombocytic mass is supposed to explain the low peripheral platelet count found in hypersplenism (43,44). Contrary to what might be expected

there is even evidence of an increased platelet production and an increased total body platelet count (45).

As far as concerns the white blood cells their quantitation is much more difficult because they are not restricted to the vascular bed. The lymphocytes constitute about half of the normal spleen cell population (46) and any real pooling and sequestration has not been found (40). The granulocytes are not found to accumulate in the normal spleen whereas in very large spleens, particularly in Felty's syndrome, increased numbers of granulocytes can be found (47). A pronounced granulocytopenia is seldom seen in hypersplenic patients (43).

These pooling-hypersequestration theories have been favoured since the middle of the sixties by a majority of authors in the field of hypersplenism. In a few diseases the spleen as well as other organs of the immune system seem to produce antibodies against the patient's own blood cells. Antibodies against erythrocytes are found in autoimmune hemolytic anemia (24), against thrombocytes in the idiopathic thrombocytopenic purpura (48) and against granulocytes in Felty's syndrome (49).

Another concept, the humoral theory, was proposed by Dameshek in 1955 (34). Using the methylcellulose-induced hypersplenism model one author claimed to have demonstrated a humoral factor to occur in the milk from lactating hypersplenic rats causing anemia in the suckling baby rats (38). Others have claimed the existence of a humoral factor in urine from hypersplenic rats causing anemia and thrombocytopenia in normal rats (39). These findings have still not been confirmed by other researchers.

The splenic-dependant tetrapeptide "tuftsin" first described in 1973 (50) stimulates the phagocytic polymorphonuclear neutrophils (28), but does not influence on the number of peripheral blood cells. As no other splenic humoral factor up to now has been identified the humoral theory cannot for the present be accepted.

The final proof for existence of hypersplenism is that removal of the spleen (or minimizing of the splenic function) is followed by an improvement of the hematologic derangement. The indications for such measures have throughout the years broadened due to the decrease in immediate operative risks (7,4 % overall mortality), (51) but this development is now partly counteracted by the increasing awareness of postsplenectomy infections.

Indications for splenectomy

An overwhelming amount of evidence speaking in favour of preserving splenic function as far as possible during surgery has accumulated since the first report of Morris and Bullock in 1919 (10). The risk of postsplenectomy sepsis was first discovered in children (13) but

later on also splenectomized adults were found to be more susceptible to bacterial infections, especially from capsulated organisms (14, 15). A great number of experimental studies have supported this concept (52,58). The injured spleen should if possible be kept in place, sutured or, if necessary, partially resected rather than removed (59,60). Splenic artery ligation or embolic therapy in order to preserve parts of the parenchyma have also been discussed (62,63) as has autologous splenic transplantation (56,58). Thus, many efforts are made to preserve the spleen after trauma; the question then arises at what point does hyperfunction indicate splenectomy.

The presence of a malignancy in the organ is, of course, an ultimate indication for splenectomy. In staging lymphomas, especially Hodgkin's disease, splenectomy has become a routine since 1969 with the aim of estimating involvement of the spleen as accurately as possible (64). In this way planned radiation towards the splenic area can be avoided (65). Furthermore, splenectomy is said to increase the tolerance to radio-chemotherapy (66) and to improve cytopenia in advanced lymphoma-diseases (67). All these statements should probably be re-evaluated. Askegren reported that early diagnostic splenectomy does not favour the prognosis in those HD patients who present uncertain prognostic indices and are therefore treated with total nodal irradiation (68). Furthermore, the improved tolerance to radiation after splenectomy has been questioned (69,70). Against these uncertain gains the increased risk for overwhelming splenectomy infection (500-fold) should be set up (11). Attempts to stage HD with a partial resection of the spleen has been abandoned as about 10 % of involved spleens are missed (71).

A "blind", hypersequestering spleen in hereditary spherocytosis is an absolute indication for splenectomy (72,73,74).

In autoimmune thrombocytopenia or hemolytic anaemia the spleen plays a double role as the source of antibody production as well as the dominant organ for cell destruction (74). About 75 % of the cases which can be identified by demonstration of the exact site of the cell destruction (75) will benefit from reduction of the spleen or splenectomy (24,72,74); in these cases removal of the whole spleen gives the best and most long-lasting results (76).

The chronic leukemias often end up with a very large, hyperfunctioning spleen giving the patient not only pancytopenia but also mechanical problems from the size and weight in the spleen. Such big spleens should be removed. The procedure is safer and easier if the splenic artery is ligated as an initial step in the operation (77). If performed early in the course of the disease there is some evidence that much of the tumor burden originating from blastic transformation (78) can be removed through splenectomy (79) in chronic myelocytic leukemia (CML) and in hairy cell leukemia. Myelofibrosis, besides CML, causes the greatest enlargement of the spleen and frequently "the spleen in question has become more harmful than beneficial" (24) making the decision of splenectomy easy.

Very seldom, a spleen enlarged due to infection has to be removed. One exception is infection in splenomegaly combined with leukopenia and rheumatoid arthritis in Felty's syndrome. In addition to the increased pooling of granulocytes (80) the spleen has been reported to be involved in production of antigranulocyte antibodies (49).

Chronic renal failure is often followed by chronic antigen stimulation resulting in a hypersplenism with, above all, lymphoid hyperplasia. The antigenic stimulation is caused by sepsis, multiple transfusions and hepatitis (81,82) and frequently results in progressive anemia with further demands for transfusion. Such patients seem to benefit from a reduction of the hyperfunctioning spleen which improves the hematologic status and reduces the need for transfusions, thus, increasing the possibility for the immunosuppressive treatment to maintain renal homografts (81,82,83).

In the infiltrative diseases splenectomy is more questionable since the drawbacks of the operation do not seem to be compensated sufficiently by improvement of the peripheral blood picture (84).

Hypersplenism due to portal hypertension caused by liver cirrhosis, portal or splenic vein thrombosis, is said to be triggered by congestion. However, the size of the spleen and the severity of the hypersplenism are poorly correlated to the portal pressure, or the degree of obstruction to the portal or splenic venous flow (85,86). Furthermore, in animal models and also in cirrhotic humans (86,87) an increased and not a diminished splenic arterial flow is found to correlate well to the spleen size. Assessments in cirrhotic patients have shown a contribution to the portal system via the splenic artery of more than 1.0 l of blood per minute (85,88,89). This fact together with a sometimes pronounced pancytopenia, especially low platelet count, has rendered splenectomy alone or as an additive treatment a frequently advocated procedure in patients with portal hypertension. However, the operation in these patients can be difficult to perform, exhibiting a mortality of 7-12 % in direct connection with the surgery (51,90) and a considerable morbidity in remote postsplenectomy infections (31,91).

Partial reduction of the splenic parenchyma in hypersplenism

In some diseases combined with hypersplenism a partial more than a total splenectomy might be beneficial; these are cases of "work hypertrophy" resulting from a hyperimmune response such as in Felty's syndrome and chronic renal insufficiency or from increased work-load infiltration such as Gaucher's disease and sarcoidosis. In congestive hypersplenism due to portal hypertension a partial reduction resulting in eusplenism will improve the peripheral blood count as well as decrease the portal blood flow and thereby lower the portal pressure (92,93).

Splenic artery ligation was performed in humans already in 1918 by

Blain (94) who summarized his experience in 1950 (95). The method was considered to reduce hypersplenism, especially in portal hypertension, with lesser risks than splenectomy. The operative mortality was lowered from 10-15 % in splenectomy to just 2-3 % in artery ligation (96,97,98). The pathophysiological changes following splenic artery ligation and the rationale for its use were studied only recently. Experimental and clinical studies have shown a close correlation between the magnitude of splenic arterial flow, the size of the splenic artery and the degree of splenomegaly (85). It has been convincingly shown that portal hypertension and splenomegaly are results of a combination of obstruction to the portal flow and a hyperdynamic blood flow (85,86). Moreover, few histologic changes of the spleen are found in dogs after splenic artery ligation (99). Ligation of the artery thus seemed to be a means of diminishing the inflow via the spleen to the portal system without much change of the splenic architecture. The first evidence for this theory was given by surgeons interested in trauma, who reported a beneficial effect of splenic artery ligation on bleeding from the traumatized spleen and few adverse effects (59, 61,62). After extensive animal experiments (100,101,102) Witte has used the ligation in hypersplenic patients with portal hypertension (102) and later also in children with peripheral blood dyscrasia (76). The immediate results on the peripheral blood count were not long-lasting and in some cases a splenectomy became necessary.

Transarterial ischemic therapy. Studies in dogs with selective embolic occlusion of the splenic artery were reported by Chuang and Reuter who obtained diminished splenic function without side effects (103). This finding initiated animal experiments and clinical studies including transcatheter splenic embolization with a number of different materials. Steel coils, Bucrylate^R (isobutyl-2-cyanoacrylate), Ivalone^R (polyvinylalcohol) and balloon catheters were used to produce total occlusion of the splenic artery. However, analogous to splenic artery ligation, only a temporary effect was observed, due to the development of collaterals (104-107). Gelfoam particles, silicone spheres, and autologous blood clots were introduced to occlude the arterial branches more peripherally, leading to partial infarction of the spleen without development of collaterals (108-111). Following several clinical reports on arterial embolization of vascular lesions in other organs (113-116), Maddison in 1973 used this method for treatment of hypersplenism in a cirrhotic patient (110). We employed similar treatment two years later in a severely ill patient. Under protection of antibiotics we used repeated treatment with small particles of gelfoam, which produced a reduction of the splenic parenchyma by 40-50 %. (148).

Clinical problems in the handling of hypersplenism

This study was initiated during a period when many centres had become disappointed with their treatment of portal hypertension. For many years, the treatment was focused on the diagnosis of bleeding esophageal varices and prevention of this bleeding by porto-systemic

shunts. It became obvious that the disease was multi-symptomatic; different patients have different symptoms, and should benefit from different treatment. This is the thought behind selective symptomatic treatment (SST) of portal hypertension and its complications (112). Transesophageal sclerotherapy has provided an effective tool for acute treatment of bleeding esophageal varices (117,118). Ascites in most cases can be managed by intensive diuretic treatment (119) and, in the event of failure by peritoneal-venous-shunting (120). Encephalopathy can be reduced by avoiding porto-systemic shunts (121).

Hypersplenism is prominent in about 20 % of our cirrhotic patients. This symptom must be attacked specifically if one of the following conditions are present: (a) splenomegaly with a thrombocyte count below $60 \times 10^9 / l$; (b) the spleen contributes markedly to the portal blood flow of the spleen, i.e. the diameter of the splenic artery exceeds a certain limit at the arteriogram (92) or the oxygen content of the portal blood drops under a certain value (93); or (c) the PTP shows varices which are fed from the spleen via the short gastric vessels (122). The effect of porto-systemic shunts on hypersplenism is much debated. The results reported range from 60 % relief from hypersplenism after porta-caval shunts (123) to an increased rate of hypersplenism after the same shunt (124). The effect of the distal splenorenal shunt is also controversial varying between 40 % beneficial effect according to Hutson and Zeppa (125) and no effect on hypersplenism reported among others by our group (126). One reason for these discrepancies might be that certain investigators have given hypersplenism a liberal definition with no clinical relevance, using for example, platelet count $< 100 \times 10^9 / l$ or WBC-count $< 5 \times 10^9 / l$ as criteria. Any operation whatsoever can easily increase these values to fall within the "normal" range and there are only a few reports on longterm results.

It is obvious that splenectomy still has its place in a selective symptomatic approach to portal hypertension. The operation in portal hypertension has been looked upon with judgements varying from enthusiasm to condemnation. Few attempts have been made to clearly define the indications for the operation. When discussing its use the operation has usually been evaluated as a method which should be used generally in portal hypertension. This might explain why the conclusions are so different. The trend has been to use it in combination with other bleeding prevention methods, for example as part of a devascularization operation (splenectomy, ligation of the left gastric artery, and right and left epiploic artery). The first report from the inventors, Womack and Peters, was fairly disappointing, showing a postoperative mortality of almost 50 % and a high incidence of recurrent bleeding (127). In contrast, Hassab in Egypt reported on excellent results from more than 600 similar operations with a hospital mortality of 10 % and a rebleeding tendency in longterm follow-up of about 10 % (128). Even better results (97 % disappearance of varices and 2 % incidence of recurrent bleeding) were ob-

tained by Sugiura and Futagawa who added to the operation a trans-thoracic esophageal transection, extensive paraesophageal devascularization, selective vagotomy and pyloroplasty (129). Unfortunately these reports are difficult to compare because of lack of control groups and heterogeneity of the patient material. Thus, one study included very sick patients with bleeding varices - half of the operations were performed as an emergency procedure (127), Hassab (128) operated upon non-cirrhotics, mainly patients with bilharzia, and the third study (129) suffers from lack of details in the patient description. The three reports clearly pointed to the often seen reluctance of successful medical innovators to use control groups. All these studies need to be redone in prospective randomized trials before any solid conclusions can be made.

The operative risks in splenectomy increase manifold if the patient presents with cirrhosis, combined with an increased bleeding tendency due to low platelet count and high pressure in the portal system and portal-systemic collaterals. This fact and the possibility that OPSI may develop are powerful arguments against total removal of the spleen in cirrhosis. It is obvious that a less invasive technique aimed to reduce the splenic function is highly desirable.

The technically most simple operative procedure to give reduction of hypersplenism is splenic artery ligation. Experimental studies have shown that the normal spleen after splenic artery ligation still has the capacity to clear the blood from damaged blood cells and particulate antigens. Furthermore, the humoral immunity measured as response to sheep erythrocytes (100) or as capacity to survive a pneumococcal challenge (58) seems still to be sufficient if a third or more of the spleen is left intact (130).

To my knowledge, only Witte et al and Zittel et al have, performed splenic artery ligation in hypersplenic rats; both groups reported that part of the splenic mass was preserved and a regression of the pan-cytopenia was obtained (101,131). Both groups used "total splenic artery ligation" obtaining similar results with ligation of the artery close to the hilum (Witte et al) or near to the coeliac axis (Zittel et al). Clearance capacity or immunologic function of the splenic remnant has up to now not been studied although several authors have stressed the great need of such investigations.

The clinical reports on splenic artery ligation have all shown good immediate results on the hypersplenism but a fairly high rebleeding frequency, e.g. up to 54 % in the Mayo Clinic series (96). In the series of Witte et al, a second operation had to be performed in about half of the patients because of recurrence of hypersplenism (102). This was probably due to development of collateral regeneration of the splenic remnant (58). Although, none of the clinical studies included control groups, the ligation showed obvious advantages over splenectomy as far as mortality and morbidity and incidence of OPSI are concerned. The longterm recurrence of hyper-

splenism, the difficulties of predicting the degree of splenic reduction obtained after ligation and the fact that splenic artery ligation demands a laparotomy have made us prefer embolization in portal hypertension.

As seen from the review of literature presented in Table A concerning transarterial ischemic therapy, many different methods have been devised in order to obtain reduction of splenic parenchyma. The total infarction obtained by using materials such as Ivalone^R, Bucrylate^R, and steel coin or balloon catheters seems very similar to splenic artery ligation. These methods can be used to facilitate a subsequent splenectomy, but even in such cases disastrous results with septic shock and fatal abscesses may occur (109). In one case reported by Günther et al (107) Bucrylate caused a total necrosis of the gastric mucosa with a fatal outcome. Also Gelfoam has given serious complications, when used to produce total infarction; these complications have been mainly infections e.g. abscesses of the spleen, pneumococcal sepsis and pneumonia. The reversal of the flow in the splenic vein due to the complete interruption of the arterial splenic flow might facilitate the development of abscesses (99,102). The best results have been obtained when a partial reduction of the splenic mass was attempted by the use of Gelfoam under antibiotic cover as described in the large series by Spigos and the smaller series by Gerlock, both concerning patients with renal insufficiency (83,132). Spigos and Gerlock embolized about 70 % of the parenchyma with good results whereas Vujic using the same method embolized 50 % of the spleen in cirrhotics with fatal results in all three cases (133). These divergent results indicate the need for further studies of these methods (I).

Long-term follow-up studies after partial reduction of the splenic mass by embolization procedures are still missing. Spigos has so far only given the immediate results in his studies (83,111,134). Such long-term follow-ups should include repeated angiograms or scintiscans in order to follow the regeneration of the infarcted spleen, as well as repeated assessments of platelet count to follow the hypersequestering capacity (99,102).

Table A

Summary of the literature on splenic artery embolization.

Author	Material of embolization	Percentage of embolized spleen	Indication	No. of pat.	Ab.	Complications	Mors
Maddison (1973)	autologous blood clots		bleeding from oes. varices	1	-	-	-
Bücheler et al. (1975)	fibrin foam	60-80 %	bleeding from oes. varices	2	-	hepatic coma 1	+
Goldstein et al. (1976)	gelfoam + steel coils	100 %	hypersplenism	2	-	abscess 1 pain, pleural effusion 1	+
Witte et al. (1976)	gelfoam balloon cath.	90 %	hypersplenism liver cirrhosis	3	-	abscess 1 abscess, pneum. 1 pneumonia 1	+
Papadimitriou et al. (1976)	gelfoam	80 %	hypersplenism liver cirrhosis	1	-	intest. paraly- sis, pain, pleu- ral effus. 1	+
Castaneda-Zuniga (1977)	polyvinyl alcohol (Ivalon ^R)	100 %	thrombocytopenia ^x	3	-	septic shock 1 abscess 1 pleural effus. 1	+
Conroy et al. (1978)	gelfoam	100 %	hypersplenism ^x Hodgkin's dis.	1	-	-	-
Wholey et al. (1978)	steel coils balloon cath.	100 %	hypersplenism	3	-	abscess 1 splen. rupture 1	-
Levy et al. (1979)	steel coils	100 %	hypersplenism ^x	1	-	-	-
Pereiras et al. (1979)	gelfoam	50 %	unknown	7	+	-	-
Zimmerman et al. (1979)	gelfoam	100 %	hypersplenism portal hyper- tension	3	-	abscess 1	-
Günther et al. (1980)	butyl-2-cyano- acrylate (Bucrylate ^R)	100 %	bleeding from oes. varices	1	-	necrosis of gastric mucosa 1	+
Spigos et al. (1980) [incl. Spigos (1977), Spigos (1979)]	gelfoam	60-70 %	hypersplenism renal insuff. 35 thalassemia maj. 5 spl. v. thromb. 1	41	+	abscess 1 pancreatitis 1 pleural effus. 2 pneumonia 5	-
Vujic et al. (1981)	gelfoam	50 %	hypersplenism liver failure	3	+	pneum. sepsis 1 abscess 1 pulm. emb. 1	+
Gerlock et al. (1981)	gelfoam	60-80 %	hypersplenism renal insuff.	6	+	-	-

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^xPreop. treatment

Experimental hypersplenism

Attempts to produce portal hypertension or congestive splenomegaly by obstruction of the portal or splenic venous flow have failed (87, 135). In 1942 Hueper produced massive splenomegaly in rabbits, dogs and rats by repeated injections of macromolecular polymers (136). This method was improved and adapted for the study of hypersplenism by Palmer et al 1953 (137). They found 15 weeks of administration of methylcellulose into rats to result in splenomegaly with anaemia, leukopenia and thrombocytopenia which all normalized when the enlarged spleen was removed. During the following years this method has been used extensively in laboratories all over the world. The studies have concerned e.g. the mechanisms involved in the development of hypersplenism (36-42,44,45) including the determination of cells, plasma and whole blood volumes in hypersplenic rats (138). Furthermore, methods for treatment of hypersplenism have been tested in this model (100,131).

As previously mentioned the spleen has several immunological functions, which are well illustrated by the effect of splenectomy, viz decreased serum IgM-levels, altered serum opsonic function and diminished response to intravenous particulate antigen immunization (31). These aspects have been studied in humans as well as in animal models; in particular the splenectomized rat model has demonstrated the importance of the spleen in affecting clearance of especially pneumococci upon intravenous injection (52-57). There is evidence that patients with alcoholic cirrhosis are unusually susceptible to infections (139). A recent editorial article in Lancet stressed the complexity of the mechanisms underlying susceptibility to infections in cirrhosis, and pointed out the need for a deeper exploration of the splenic function in such cases (140). Little attention, however, has been paid to the susceptibility of hypersplenic individuals to infections. A survey of the literature (Table A) reveals many problems concerned with infections during treatment of hypersplenism. For example two cirrhotic patients referred to in Table A died from septic shock and in one of them reported by Vujic (133) the death was proven to be caused by pneumococcal infection. As there is strong evidence of a similar pathogenesis and splenic histopathology of the large spleen in hepatic cirrhosis and blood dyscrasias associated with work hypertrophy (86) the experimentally methylcellulose-induced hypersplenism might be a suitable model for studying these problems(III).

Only two experimental works have been published concerning hypersplenic animals treated by partial reduction of the splenic mass. Both papers employed methylcellulose-injected rats and both used splenic artery ligation (101, 131). Neither of these reports mentioned infection problems, quite contrary to what might have been expected from clinical experiences of splenic artery ligation and embolization of the splenic parenchyma. Thus, in the clinical cases abscess formation seem to be frequent, often leading to death (Table A). These problems have been studied in paper IV.

Hypersplenism and hemostasis

As already mentioned hypersplenism is defined as an enlargement of the spleen combined with anemia, leukopenia, trombocytopenia or any combination of the three. Depending on the underlying disease the presently available symptomatic treatments consist in administration of glucocorticoids, antibiotics or blood component transfusions. Subnormal values of platelets are often considered to be of minor clinical importance but occasionally the trombocytopenia can be a real threat to the patient requiring large amounts of platelet infusion (72, 141). In other cases the trombocytopenia results in only small cutaneous gingival or occult gastrointestinal bleeding (petechiae). It is a well-known clinical experience that the severity of the bleeding tendency is impossible to estimate from the platelet count. The same discrepancy has been noticed by many surgeons performing splenectomies because of hypersplenism; on one occasion no obvious bleeding tendency was present despite low platelet values (77), and in another the opposite situation was encountered. A clinical observation is also the very prompt improvement of the bleeding tendency after splenectomy.

As shown by Zoucas and colleagues the platelet function is well estimated by measuring the aggregability of the platelets and the bloodloss and bleeding time after a standardized operation (142). Since the primary aggregation is of particular interest in the acute operative situation, rats in which no secondary aggregation occur seem to be suitable for such studies (143).

AIMS OF THE PRESENT INVESTIGATIONS

The aims of the present studies were

1. To evaluate partial splenic embolization as an alternative to splenectomy in hypersplenic patients with liver cirrhosis (I).
2. To investigate in experimentally hypersplenic rats the effect of partial reduction of the splenic parenchyma on the peripheral blood count and the infection complications in the surgical procedures (II).
3. To investigate the susceptibility of experimentally hypersplenic rats to pneumococci (III).
4. To elucidate the effect of splenic artery ligation in experimentally hypersplenic rats with special reference to the defence against bacterial infection (IV).
5. To elucidate the hemostasis in the hypersplenic state (V).

MATERIAL AND METHODS

The clinical study (I)

Twenty-five patients with portal hypertension and hypersplenism were treated on 49 occasions with splenic embolization via the splenic artery. The patients were 30-73 years of age with a median age of 64 years. All the patients, except one, had experienced at least one episode of oesophageal varix bleeding diagnosed by acute endoscopy. Nineteen patients contracted varices due to liver cirrhosis; two patients were classified as belonging to Child group A, ten to group B and seven to group C (144). Among the remaining patients, two had developed congestive hypersplenism due to splenic vein thrombosis (regional portal hypertension) and four patients due to portal vein thrombosis (table I). With the exception of the individuals who suffered from regional portal hypertension all patients were thrombocytopenic ($<60 \times 10^9$ platelets/l), a pre-requisite for including them in the study. In the patients with splenic vein thrombosis the indication was to reduce the splenic blood flow. In addition to routine blood examinations, antibody assessments, coarse needle biopsy of the liver, coeliac arteriogram and percutaneous transhepatic portography (PTP) were performed in all patients.

The embolization was performed with an angiographic catheter introduced via the femoral artery as distally as possible into the splenic artery. Small pieces of Gelfoam soaked in Keflin^R solution were injected through the catheter to cause occlusion of the small distal splenic artery branches. The extent of the splenic infarction was estimated from intercurrent angiograms and the treatment was, in general, finished after 30 - 40% infarction was obtained. The day before embolization systemic oral administration of cephalosporin (0,5 g x 4) was started and continued for 2 weeks.

After the initial embolization the patients were followed according to a fixed scheme including liver tests and repeated assessments of peripheral blood elements, regular scintiscans and in some cases new angiograms, together with registration of complications, e.g. bleeding from the varices. Additional treatment such as PTP or trans-oesophageal sclerosing treatment was also registered. If the result of the first embolization was judged to be unsatisfactory, i.e. the bleeding tendency persisted, or unaltered low values of platelets were seen, the embolization was repeated up to two times with intervals of 2 months in order to obtain in selected cases a maximum infarction of up to 70-80% of the initial splenic parenchyma.

The experimental studies (II-V)

Animals

Male, albino, Sprague-Dawley rats were used. The rats were housed 5 per cage and kept under ordinary laboratory conditions. They were

given standard food pellets and water ad libitum. All operations were performed under light ether anesthesia and under clean conditons.

Induction of hypersplenism (II-V)

According to the method described by Palmer et al (1953) the rats, initially weighing about 225 g were given 2 ml of 2,5 % methylcellulose solution intraperitoneally twice weekly for 12-14 weeks. Control rats were given 2 ml saline (0.9 g/l) following an identical scheme. The progress of hypersplenism was followed by repeated determination of hemoglobin concentration, white blood cell count and platelet count. The weight of each rat before and after treatment and, if possible, the weight of the spleen after methylcellulose administration were also determined.

Preparation of the methylcellulose solution (II V)

The procedure followed the original description by Palmer et al (1953). Methylcellulose of viscosity grade 400 centripoises (Methocel, A4, Dow Chemicals, 2160 Stade-Brunshausen, West-Germany) was obtained as a dry white powder. The powder, 2,5 g, was dissolved in 100 ml distilled water at a temperature of 80° C. This mixture was then cooled resulting in a clear viscous solution. The methylcellulose solution was kept in a refrigerator until used and repeated cultures were taken to exclude bacterial contamination.

Surgical procedures

The various operations on the spleen were performed through a midline abdominal incision. A reduction of the splenic parenchyma was attempted by one of the following methods:

1. After ligation of the corresponding vessels using 6/0 silk, approximately the lower third of the spleen was resected. A catgut ligature was placed around the spleen and near to the cut surface to obtain hemostasis (paper II).
2. The same procedure as above but 2/3 of the spleen was resected (II).
3. Total splenectomy (II).
4. The vessels estimated to supply the lower third of the spleen were ligated and divided close to the spleen, leaving the organ in situ (II).
5. Same procedure as above but vessels supplying the lower two thirds were divided (II).
6. All splenic vessels were ligated and divided close to the spleen (II).

7. In some control animals the spleen was mobilized and put back at its site (sham-operation) (II).
8. Splenic artery ligation was performed using a Zeiss OPMI 6-S operative microscope. Via a midline incision the splenic artery was separated from the splenic vein and ligated just proximal to the branching of the upper splenic pool artery using silk 6/0 (IV).
9. As a control group to the splenic artery ligated rats the splenic artery was dissected but not ligated (IV).

Challenge of rats with pneumococci (III-IV)

Streptococcus pneumoniae type I were prepared and stored at -80°C (57). Just before use, the pneumococci were thawed and then immediately injected intravenously into the hind limb or the penile vein of the animals. All rats that died were autopsied and the blood cultured. Surviving animals were killed after two weeks, dissected and blood taken for culture.

Determination of peripheral blood cell count (I-V)

In the clinical study (I) standard methods according to the chemical laboratories of Lund hospital were used for determination of hemoglobin concentration, hematocrit (Hct), white blood cell (WBC) counts and platelet counts.

In the experimental studies the hemoglobin concentration in the animals' blood was determined photometrically according to Kampen and Zijlstra (145). The Hct was determined in duplicate by the micro-hematocrit method. WBC counts and platelet counts were determined in a Bürker chamber using a phase contrast microscope.

Determination of bleeding time and blood loss (V)

After a standardized resection of the left anterior liver lobe the blood was collected and weighed and the bleeding cut surface was observed through an operation microscope in order to determine the bleeding time. The method is described in detail in reference 142.

Determination of APT-time (V)

Using a commercial standardized reagent containing a soluble activator (Cephotest, Nyegaard, Denmark) the APT-time was registered according to Proctor and Rapaport (146).

Platelet aggregation assay (V)

The assay was performed according to the method described by Born and Cross 1963 (147) using plasma containing $650-700 \times 10^9$ platelets/L.

Adenosine diphosphate (ADP) in final concentrations of 0.1, 0.2 and 1.0 $\mu\text{mol/l}$ and collagen in final concentrations of 1% and 2% w/v , respectively, were used as aggregating agents.

Statistical analysis

Student's t-test for unpaired data was used to evaluate the effect of methylcellulose and to compare the outcome of the different operations (II).

Student's t-test for paired data was also used to evaluate the effects of the treatments (II, III, IV).

The chi-square test (exact method) was used to evaluate the outcome of pneumococcal challenge (III, IV), and the same test with Yates's correction for small samples to compare the hemostatic effects of hypersplenism (V).

RESULTS AND DISCUSSION

The clinical study (I)

The first clinical embolization of the spleen for hypersplenism was performed by Maddison in 1973 (110); since then, about 80 treatments have been reported from more than a dozen different groups (Table A). It appears from these papers that gelfoam gives the best results and that the lowest complication rate is obtained if no more than 70 % of the splenic parenchyma is embolized. Spigos and his group, who have collected most of the experience with this method, have treated above all patients with chronic renal insufficiency (83) whereas our group has mainly focused on hypersplenism secondary to portal hypertension. Our initial data were reported in 1979 (148) and the latest results are given below.

In general, the patients' mean WBC count and mean platelet count rose to normal values within one week after the first treatment whereas the mean hemoglobin concentration remained at the subnormal pre-treatment value. Eleven patients could be followed for a longer period; the platelet count showed after half a year values within the normal range. However, after two to three years a decline to sub-normal values in the number of thrombocytes was registered, in three patients. (Fig 1 5). The overall number of bleedings from the oesophageal varices was reduced and in seven patients no bleeding at all was seen after the embolization. It should be noted, however, that the embolization was most often performed in connection with another sort of treatment as a part of our SST program. The course of the disease in these patients is shown in Fig 1 6.

Eleven patients died within half a year after the first partial embolization. In three of these patients deaths were judged to be related to complications after the treatment, resulting in a mortality rate of 6 % (3 of 49 treatments). One patient contracted an abdominal abscess following total splenic infarction; the second, in the final stage of liver cirrhosis, died from pneumonia; and the third rebled, showing pleural effusion. The remaining fourteen patients survived for more than four years after treatment and some are still alive, > 6 years after the first treatment. One patient died from liver insufficiency following mesocaval shunting after variceal bleeding 4.5 years after treatment. Two patients died from malignancies, one from pancreatic carcinoma after experiencing five years of freedom from bleeding after the embolization; the other patient died from nephrocarcinoma after having experienced one minor bleeding episode during the 4.5 years after the splenic embolization. Side-effects and complications to the embolization procedure in our patients are listed in Table I B.

Cirrhosis of the liver is often associated with splenomegaly (75 %) and sometimes also with thrombocytopenia (16 %) or leukopenia (20%) fulfilling the criteria for hypersplenism (149). The mechanism

behind the development of the hypersplenism is considered to be congestion and the size of the spleen is correlated more to the arterial blood flow than to the level of the portal blood pressure (85, 86). Obstruction of the portal or splenic vein blood flow is not sufficient to produce congestive splenomegaly in laboratory animals (135). Patients with splenomegaly resulting from hepatic cirrhosis have an increased and not a diminished splenic arterial flow (85, 89). Splenectomy in portal hypertension has been considered beneficial because of the reduction of the increased blood flow hereby obtained; thus, the amount of blood entering the portal system is reduced. We have until now not been able to measure the portal blood flow in any of our patients. The portal pressure has been recorded in only three patients synchronously with the splenic embolization; an immediate lowering of the portal pressure was not noticed (148).

Splenic embolization of the hypersplenic spleen has also shown good immediate effects on the peripheral blood count in smaller series (110, 111, 132, 151, 152) as well as in the large series by Spigos et al (83). Our long term results, however, showing a decline in the platelet count and increase in the size of the embolized spleen (as seen at scintiscan) might be ascribed to the capacity of the spleen to regenerate (153-156). The effect of embolization on recurrent bleeding is more difficult to evaluate since oesophageal sclerosing treatment and, in two cases, portocaval anastomosis, were performed together with the partial splenic embolization. Whatever the reason, the bleeding tendency, however, markedly decreased and half of the surviving patients did not rebleed. The fact that the recurrent bleedings often occurred more than two years after the last embolization, together with the increase in spleen mass at that time demonstrated the importance of the embolization and indicated that the procedure should be repeated after two to three years (Fig I 5).

The frequency of serious complications such as intra-abdominal abscesses, splenic vein thrombosis and pneumonia was 7/49 treatments (14%): this should be compared with 27 % compiled by Fabri in his large series of splenectomies (51). Also, the mortality, 6 % is well below the 13 % reported by Fabri. The fairly high rate of complications in our study must be set in relation to the severity of the disease in the patients, most of them belonging to Child groups B and C. Hence, in some of the cases the method was used as the last possible treatment. However, treatment performed in the later part of the study in which a smaller part of the spleen was embolized during each treatment has resulted in fewer serious complications. The immediate pain, more a result than a complication of the method, and lasting for not more than two or three days, was comparatively mild and easily relieved with mild analgetics. Narcotic drugs or epidural anesthesia as used by Spigos (83) or intraarterial injections of chloroprocain as used by Gerlock (157) were not considered necessary. If affected, the pleura and the lungs cleared in a few days and in only one case was a thoracocentesis performed. The fever registered in 30 patients might be ascribed to a combination of unspecific

pleural and peritoneal engagements and the presence of infarcted splenic parenchyma. These mechanisms might also explain the prompt increase in WBC-count. A supernormal value of S-amylase was noticed in half of the patients but no case of pancreatitis giving clinical symptoms was observed. Spigos reported one case of pancreatitis (83) probably due to inadvertent embolization of pancreatic arterial branches. This complication was probably avoided in our study because the tip of the catheter was placed as distal as possible in the splenic artery.

In conclusion, partial embolization, performed stepwise with gelfoam and under antibiotic cover was found to be a good means of selective symptomatic treatment of hypersplenism as a complication to portal hypertension.

The experimental studies (II-V)

Several studies have indicated the importance of leaving a certain part of the spleen functioning after a partial embolization; this seems to be critical for the defense against infections with encapsulated bacteria. The rat model, in which hypersplenism is induced by repeated injections of methylcellulose proved to be useful in studies of the susceptibility of hypersplenic individuals to challenge with pneumococci and the effect of splenic artery ligation on this susceptibility. The methylcellulose treated rat also constituted a suitable model for investigation of the role of thrombocytes in the hemostasis by hypersplenism.

Effect of methylcellulose treatment on peripheral blood counts

The 14-week methylcellulose treatment resulted in a significant splenomegaly combined with a decrease in hemoglobin concentration and platelet count. No decrease in body weight of the treated rats was registered as compared with rats given saline. The WBC-count did not decrease significantly except in the hemostasis study (V). The reason for these discrepancies is at present not known. The results from the four experimental studies as far as regards the blood cell components are shown together in Table B.

Table B

Effect of methylcellulose treatment to rats for 12-14 weeks on weight of rats, weight of spleens and peripheral blood elements as compared with saline administration. (Values are given as mean $\pm$ standard error of the mean.)

Study	Treatment	No. of rats	Weight of rats	Weight of spleens	Hemoglobin (g/l)	Hematocrit (%)	White blood count (10 ⁹ /l)	Platelet count (10 ⁹ /l)
II	methylcellulose	77	444 $\pm$ 4.6	5.28 $\pm$ 0.30 (n=12)	145 $\pm$ 2.0		12.7 $\pm$ 0.43	642 $\pm$ 12.6
	saline	13	463 $\pm$ 9.1	0.96 $\pm$ 0.04 (n=5)	167 $\pm$ 2.1		11.0 $\pm$ 0.95	824 $\pm$ 25.7
III	methylcellulose	80	425 $\pm$ 6.6	8.05 $\pm$ 0.34	118 $\pm$ 1.3	34 $\pm$ 0.4	10.5 $\pm$ 0.55	529 $\pm$ 12.3
	saline	20	417 $\pm$ 6.1	0.96 $\pm$ 0.03	162 $\pm$ 2.3	46 $\pm$ 0.6	11.0 $\pm$ 0.74	896 $\pm$ 39.5
IV	methylcellulose	12			108 $\pm$ 2.6	33 $\pm$ 0.9	9.8 $\pm$ 0.64	338 $\pm$ 16.2
	saline	10			163 $\pm$ 2.0	44 $\pm$ 0.8	10.4 $\pm$ 0.95	820 $\pm$ 31.8
V	methylcellulose	16		7.8 $\pm$ 0.30	99 $\pm$ 2.1	38 $\pm$ 1.8	8.2 $\pm$ 1.50	580 $\pm$ 22.8
	saline	16		0.8 $\pm$ 0.01	125 $\pm$ 1.9	50 $\pm$ 2.1	14.4 $\pm$ 1.01	987 $\pm$ 36.1

The "Palmer-model" has been used by many investigators and all found increasing spleen size with marked changes in peripheral blood cell counts following the methylcellulose treatment (36,45). The original paper (137) reported a more pronounced change in the WBC-count whereas the platelet count was unaltered. The discrepancy in platelet count as compared with, for example, our studies could possibly be ascribed to the fact that Palmer et al only obtained a splenomegaly of about three times the normal spleen size whereas we obtained an 8-fold increase in the splenic weight.

Hypersplenism and infection (I-IV)

In two patients in the beginning of our series of splenic embolization a total infarction of the parenchyma was registered. Both of them developed abscesses of which one proved to be fatal. The non-fatal abscess showed growth of α -streptococci. Another patient in the same series acquired pneumonia leading to his death. Hence, infections seemed to be one group among the serious complications in this procedure.

Among the rats in study II five animals died during the initial methylcellulose treatment, probably from respiratory tract infections although cultures did not reveal any potentially pathogenic bacteria. After the different operations 21 more rats died, 8/12 in the group in which "total ligation of the artery" was performed (Fig II 1). In three rats cultures from abdominal abscesses revealed growth of E. coli and in two rats Streptococcus faecalis was found. The significance of these cultures is difficult to evaluate as they were taken several hours after the death of the rats.

Although infections have not been reported previously in connection with methylcellulose-induced hypersplenism, many clinical papers report on great difficulties to avoid such complications after reduction of the splenic parenchyma especially when the greater part of the spleen is infarcted (105,106,109).

The asplenic individual is known to run a high risk of OPSI especially caused by encapsulated bacteria (10-15).

Against the background of the experiences referred to above, we decided to investigate whether an increased susceptibility to pneumococci also might be present in hypersplenism. For this purpose, we used frozen standardized inoculi of the bacteria (57).

Among the hypersplenic animals in study III, 7 of 20 died if given 4×10^5 CFU of Streptococci pneumoniae type 1, and 8 of 20 died if given 4×10^6 CFU. Hypersplenic rats given 4×10^4 and saline treated rats given 4×10^6 CFU of the same pneumococci did not differ in survival rate (Table III A). Blood taken from the animals that died all grew pneumococci whereas blood from the 83 survivors was culture negative in all but one animal.

As reports on susceptibility to infections in hypersplenism are not available in the literature there are no data for comparison. Obviously, the methylcellulose-treated rats were more susceptible to pneumococci than were normal rats, as 4×10^5 CFU killed 7 of 20 hypersplenic rats whereas the normal rats cleared 4×10^6 pneumococci from the blood. Comparison of these data with our previous report (57) demonstrate that one thousand of the number of pneumococci killing 7/20 hypersplenic rats was lethal for splenectomized rats. Furthermore, the survival time in the splenectomized group was 36 hours (range 35-132) as compared with 90 hours (range 43-134) in the hypersplenic group. Obviously, the methylcellulose injected rats succumbed to a more prolonged septicemia indicating that although the resistance of the hypersplenic rats to pneumococci was impaired, it was not decreased to such a degree as seen in the splenectomized rats.

The reason for the increased susceptibility of the hypersplenic rats is not fully understood. One explanation might be that the macrophages of the spleen were blocked by the methylcellulose. Another possibility could be impaired production of tuftsin but little is known about the production of this substance in hypersplenism. Tuftsin binds to polymorphonuclear neutrophils and stimulates their phagocytic effect (28). A defective phagocytosis with a high incidence of infections has recently been demonstrated in splenectomized patients and individuals with congenital tuftsin deficiency (29).

We concluded that the hypersplenic rats were more susceptible to pneumococci than were normal rats. The possibility that hypersplenic patients might have an impaired resistance to infections with encapsulated bacteria should also be considered.

The effect of ligation of the splenic artery was also studied in experimentally hypersplenic rats (IV). The operation normalized the platelet count, increased the WBC-count to beyond the upper normal limit and significantly lowered the hemoglobin values (Table IV A). The administration of 4×10^6 CFU of pneumococci killed 11 of 12 animals subjected to splenic artery ligation, whereas all of the 10 sham-operated animals survived (Table IV B). All rats that succumbed, died within 30 hours (median 17 h) after pneumococcal challenge. Heart-blood from all killed rats grew pneumococci, while blood taken from all surviving rats was culture negative. At autopsy, the splenic artery-ligated rats showed total or partial replacement of the splenic parenchyma with abscess.

The effects here obtained of splenic artery ligation (IV) on the WBC-count and platelet count were in accordance with the results in the reports by Witte et al (101) and Zittel et al (131). However, the anemia found in the rats was aggravated in our investigation in contrast to the improvement after splenic artery ligation reported by Zittel et al (131). Witte et al (101) did not report on the erythrocyte count or hemoglobin concentration. It is highly possible that the leukocytosis and anemia found in our study could be explained by

splenic abscess formation, following the ligation. The infusion of methylcellulose was continued after the artery ligation in the studies of Witte et al (101) and Zittel et al (131) as well as in our investigation.

Cooney et al (58) found splenic artery-ligated normal rats to be less susceptible to pneumococci than splenectomized rats though more susceptible than normal animals. Thus, the ligation in itself does not seem to cause total derangement of the splenic defence mechanisms.

In conclusion, ligation of the splenic artery in hypersplenic rats did not leave the resistance to pneumococci intact but caused serious side effects, i.e. formation of abscesses in the spleen.

Hypersplenism and hemostasis (V)

It is well known that serious bleeding may occur during operation in patients with hypersplenism even when the platelet count in the peripheral blood is within the normal range.

In order to study the hemostasis in the hypersplenic state, rats were made hypersplenic by methylcellulose injections which markedly decreased Hb, Hct, WBC-count and platelet count. The animals were then splenectomized. Fifteen days after the operation the Hb- and Hct-values had normalized whereas the WBC and platelet counts had increased significantly to above the upper number of the respective cells found in the methylcellulose-treated rats. Splenectomy of the control animals did not significantly affect these values (Table V B).

Bleeding time and blood loss during a standardized liver resection were significantly increased in rats made hypersplenic with methylcellulose when compared with the controls (Table V C). Removal of the hyperfunctioning spleen resulted in a normalization of the bleeding time and the blood loss during operation. The ATP-time was markedly increased in all hypersplenic rats, splenectomized or not, whether tested before or after the liver resection (Table V B). Not only the amount, but also the aggregation ability of the platelets were decreased in the hypersplenic rats compared with controls. The platelet count was increased beyond the normal values whereas the aggregation was even more impaired when the hypersplenic rats were splenectomized (Tables V E, V F). No changes could be registered in normal animals subjected to splenectomy. The data are presented in detail in paper V (Tables V A-F).

The increase in bleeding time and blood loss during a standardized liver resection in the hypersplenic animals might be explained by hemodynamic changes such as increased portal blood flow. Thus, removal of the hyperfunctioning spleen normalized the bleeding tendency as well as the platelet count. On the other hand, splenectomy in normal control rats did not affect the bleeding time or blood loss.

Hence, the increase in liver blood flow caused by the hyperfunctioning spleen was not the only cause for the pathological bleeding tendency.

This theory was also corroborated by the findings that hypersplenism significantly impaired ADP - and collagen - induced platelet aggregation. Both the number and the function of the platelets were obviously impaired in hypersplenism, also demonstrating that hemostatic mechanisms might be more important than hemodynamic changes.

An interesting finding was the inability of the splenectomy to normalize the platelet aggregation in spite of the normal bleeding time, the normal blood loss and the supernormal platelet counts found postoperatively. Splenectomy in control animals did not influence bleeding tendency, hemoglobin concentration values, hematocrit, platelet number or platelet aggregation. One explanation might be that the hypersequestering large spleen destroyed the most active, larger platelets leaving the smaller hemostatically more inactive platelets in the blood (158, 159). Removal of the large spleen in humans as well as in animals resulted in a reactive thrombocytosis (160, 161). These platelets, however, are in humans found to be functionally defect perhaps due to reduced 5-HT content and uptake (161).

The decrease of APT-time found during methylcellulose treatment is not fully understood. Large molecules such as dextran are known to prolong the APT-time due to intervention with the intrinsic coagulation system (162). Thus, need for the further studies of the hemostatic mechanisms in hypersplenism are obvious.

FUTURE ASPECTS

Paper I describes the use of partial splenic embolization in hypersplenism in patients with portal hypertension or chronic renal insufficiency. It is highly probable that this method also might have a place in the treatment of blood dyscrasias, e.g. thalassemia major, myelofibrosis and spherocytosis. Furthermore, immune thrombocytopenic purpura or immune hemolytic anemia of the "warm antibody type" might benefit not only from the reduction of the hypersequestering capacity of the spleen but possibly also from infarction of some of the immunocompetent splenic tissue taking part in the production of the harmful antibodies. In all these diseases, partial embolization might be superior to splenectomy, since the resistance to overwhelming infections is partially preserved. It remains to be seen if partial embolization might be used in selected patients suffering from leukemias or lymphomas.

The infection problems associated with hypersplenism have been noticed by several authors, and severe infections in such patients were also found in paper I. Paper II gave the experimental background to this susceptibility, demonstrating that methylcellulose-treated rats were abnormally sensitive to pneumococcal challenge. A thorough study of the frequency of septicemia in hypersplenic patients (OHSI) is highly warranted. These individuals might be candidates for infection prevention programmes, e.g. vaccination.

The experimentally obtained good results from splenectomy on bleeding tendency and operative blood loss indicate that the partial reduction by embolization could also be beneficial. In the future this could make partial splenic embolization suitable preoperative treatment in hypersplenic individuals not only before a splenectomy but also prior to any important surgery or eventually delivery.

The experimental studies (II-V) demonstrated the complex effects of the spleen in hemostasis and resistance to infections. These aspects of the splenic function are far from sufficiently elucidated. The exact mechanism by which the spleen protects against overwhelming infection is intriguing and studies of the exact role of the macrophages and humoral factors, e.g. tuftsin or other (unknown) components will probably bring about new methods for treatment of hypersplenism.

SUMMARY

The clinical part (I) of the present study showed that repeated partial embolization of the spleen might be an alternative to splenectomy in patients with hypersplenism secondary to portal hypertension. The embolization procedure improved the thrombocytopenia and substantially diminished the number of bleeding episodes. The procedure is comparatively simple, needs no laparotomy, and if performed stepwise under antibiotic cover, gives few infectious complications. The mortality registered in the study was about half of the mortality reported by others after splenectomies in cirrhotic individuals. The discomfort to the patients, e.g. pain, fever and pleural affection was mostly short-lasting and easily treated. These immediate results after the splenic embolization are in accordance with other studies using the same method. In contrast, investigators who reduced the splenic mass with more than 75 % on a single occasion have reported infectious complications giving a substantially higher morbidity and mortality. The long-term follow-up in the present study demonstrated the need for further embolizations after 2-3 years in order to preserve the "normosplenic" state, because of the regenerative capacity of the splenic remnant.

The experimental studies were all performed on rats, made hypersplenic by intraperitoneal injections of methylcellulose. The methylcellulose resulted in enlargement of the spleen and anemia and subnormal WBC-count and platelet count values as a consequence of the hypersplenism. The large spleen was reduced by partial splenectomy or ligation of the vessels supplying part of the spleen (II). One third reduction of the enlarged spleen normalized the peripheral blood picture whereas with two thirds reduction the risks for infectious complications increased, especially if infarcted splenic tissue was left behind. These findings indicated that repeated embolization, resulting in infarction of about one third of the spleen after each treatment, was superior to a single larger embolization.

The above-mentioned animal model was also used to study the susceptibility of hypersplenic rats to pneumococcal challenge (III). Using standardized, frozen inocula of Streptococcus pneumoniae the hypersplenic rats were found to be significantly more susceptible to such challenge than normal rats.

Hypersplenic rats made eusplenic by ligation of the splenic artery were found to develop abscesses in the infarction tissue (IV). Injection of pneumococci into these animals caused a significantly higher mortality than that found in hypersplenic rats given the same dose of pneumococci.

Several hemostasis parameters were also studied in methylcellulose-treated rats (V). The decrease in platelet count described above was accompanied by impaired aggregation ability of the thrombocytes. Furthermore, bleeding tendency and operative blood loss during a standardized liver resection were found to be increased in hyper-

splenism. Splenectomy normalized the bleeding tendency although improvement of the thrombocyte aggregation was not noticed.

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EVALUATION OF SPLENIC EMBOLIZATION IN PATIENTS WITH PORTAL
HYPERTENSION AND HYPERSPLENISM

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Running title: Splenic embolization in portal hypertension

ABSTRACT

Twentyfive patients with hypersplenism due to portal hypertension were treated 49 times by repeated partial splenic embolization. Three patients died in connection with the treatment and another eight patients died within half a year due to the underlying liver disease. Fourteen surviving patients were followed for up to six years showing a good response on peripheral blood count and bleeding tendency. The discomfort and complications of fever, pain, pleural effusion and abscess formation and the possibility to avoid these by repeated partial embolization under antibiotic cover are discussed. The results are compared to reports in the reviewed actual literature and the splenic embolization is given a place among the means of a successful selective symptomatic treatment of portal hypertension.

Key words: Splenic embolization, hypersplenism, portal hypertension, long-term follow-up.

Portal hypertension has four well-known complications: bleeding esophageal varices, ascites, encephalopathy and hypersplenism. In the past most of the interest has been focused on the bleeding varices and its prevention; the other complications have been more or less neglected. Today there is a tendency to a more conservative - non-invasive - approach to portal hypertension in general. This has led surgeons and physicians including ourselves to give more attention to the other complications and their treatment, selective symptomatic treatment (SST)(3). A consequence of this is the new view on ascites, which in most cases can be managed by intensive diuretic treatment (11), and if failure occurs, by peritoneal-venous shunting (28). Another consequence is the newly awakened interest in the transoesophageal sclerotherapy which has provided an outstanding tool for treatment of bleeding oesophageal varices (1, 16). It is also in line with this that alternative techniques for treatment of splenomegaly and hypersplenism are discussed (34).

It is not long since splenectomy was a hazardous procedure especially in patients with advanced portal hypertension and in patients with hematological disease (17, 30). Despite the fact that, due to good preparation and careful surgery, the splenectomy can be performed in most cases with a low mortality, the rate of complications are still considerable (30, 41). There are also cases where, due to advanced thrombocytopenia, the presence of other diseases, especially anergy or malnutrition, splenectomy is still to be considered as a risky operation (17, 38).

It is well documented through experimental and clinical studies that splenic artery ligation is an effective tool to relieve thrombocytopenia (13, 57). Due to the rapid and rich collateralization of arterial and venous communications over the gastric, gastroepiploic and pancreatic network, the effect of splenic artery ligation is unfortunately short-lasting (58). It is our opinion that it is necessary to occlude the arterial supply more peripherally, i.e. in the splenic parenchyma.

It was Maddison (31) who first performed clinical embolization of the spleen. After him during the last half of the 70's more than a dozen reports are found in the literature, some claiming success without serious complications (18, 34, 47), others describe a high incidence of serious complications (8, 19, 50, 56). As a consequence of the early experience with splenic embolization, several attempts have been suggested to reduce the complication rate. These methods include splenic embolization under prophylactic antibiotic protection as well as several step-occlusions, i.e. repetition of a careful embolization with some weeks intervals.

Our first experience with the embolization technique was described in 1979 (34). Now it is possible to summarize our long-term experience with embolization of the spleen as a part of a programme for selective symptomatic treatment of portal hypertension and its complications.

MATERIAL AND METHODS

Patients

In the period September 1975 to March 1981, 25 patients (15 women and 10 men) were at 49 occasions the objects for partial splenic embolization with the aim to eliminate hypersplenism. Nine of the patients received one treatment, eight patients received two treatments, and eight patients underwent treatment on three occasions. This in order to eliminate the complications of too much reduction at one time. The patients were in the age group of 30-73 years with a median age of 64 years (Fig. I 1). All patients except one had had at least one episode of bleeding from esophageal varices. The main etiologic factor was liver cirrhosis in 19, portal venous thrombosis in four, and splenic venous thrombosis in two (Table 1 A). Furthermore, patients with liver cirrhosis and portal thrombosis all had thrombocytopenia ($<60 \times 10^9/l$) which was a pre-requisite for including them in this study. In patients with a splenic vein thrombosis the indication was to reduce the splenic blood flow.

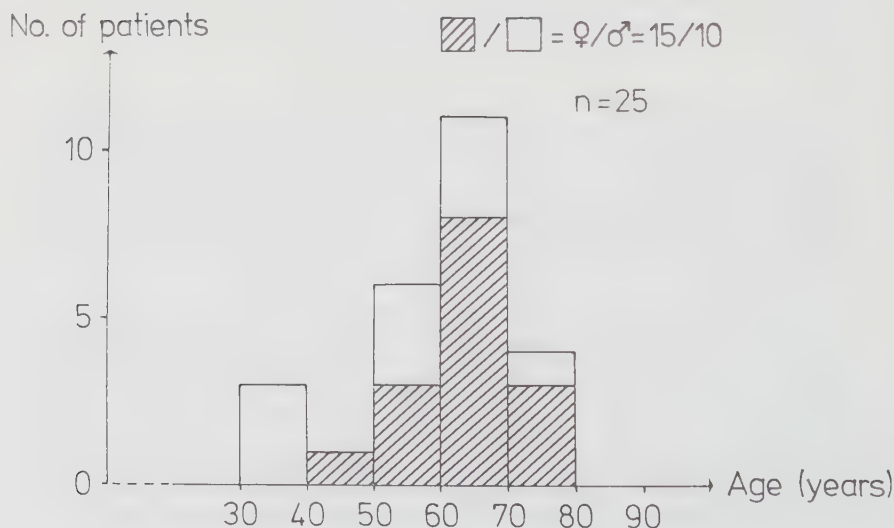


Fig I 1. Age and sex of 25 patients treated with splenic embolization.

All patients were the objects of detailed work-up before the treatment, as well as careful follow-up after the treatment. The examinations included in all patients routine laboratory tests, antibody assessments, coarse needle biopsy of the liver, arteriogram and percutaneous transhepatic portography (PTP). In some patients the size of the spleen was followed by repeated scintiscans. The patients with liver cirrhosis and portal hypertension were classified according to the Child classification system (9). Most of the cases had an advanced disease. Thus, two patients were classified as Child A, ten as Child B and seven as Child C.

Prior to the first embolization the mean hemoglobin concentration was 113 g/l (range 79-152), mean WBC count was $4.9 \times 10^9/l$ (range 2.1-12.3) and mean platelet count was $68 \times 10^9/l$ (range 30-132).

Technique

An angiographic catheter was introduced selectively into the splenic artery via the femoral artery. It was placed as distal as possible, and small pieces of Gelfoam soaked in Keflin^R solution were injected through the catheter in order to occlude the small splenic artery branches, and induce minute splenic infarction. It was our intention to induce fibrosis in 30-40% of the splenic parenchyma on each treatment, thus avoiding total splenic destruction and/or major abscess formation. On the day before the treatment a two week^R systemic antibiotic medication (cephalexin monohydrate, Keflex^R 0.5 g x 4) was started.

After the treatment each patient was followed-up according to a special protocol. White blood count, platelets, peripheral blood elements, bleeding from varices and occurrence of complications, were registered.

If the result was judged as unsatisfactory, i.e. remaining bleeding tendency, low post-treatment values of the platelets, or a combination, a new course of treatment was given after six weeks or later.

RESULTS

Discomfort and complications

After 30/49 treatments, a moderate fever up to $38.5^{\circ}C$, and usually lasting 2-5 days was noticed. In half of the patients a transient elevation of s-amylase was seen in the postoperative period, although no clinical signs of pancreatitis were observed in any of the patients. 1/3 of the patients complained of moderate pain in the left hypochondrium which was easily controlled with light analgetics. In table I B the complications are listed. Apart from fever and moderate abdominal pain, pleural effusions with or without small left-sided atelectases were the most frequent complications. They were noted on routine chest films and were

usually not followed by any clinical signs and resolved within two weeks. In one case thoracocentesis was needed. Splenic vein thrombosis was found at routine follow-up angiograms but none of them gave any clinical signs of the complication. Two of the patients showed signs of abscesses.

Table I A. Diagnoses of 25 patients with hypersplenism

Alcoholic cirrhosis	8
Primary biliary cirrhosis	2
Secondary biliary cirrhosis	2
Chronic aggressive hepatitis	2
Posthepatic cirrhosis	4
Cryptogenic cirrhosis	1
Portal vein thrombosis	4
Regional portal hypertension	<u>2</u>
	25

Table I B. Complications in 49 embolizations

Abscess	2
Splenic vein thrombosis	4
Pleural effusion and/or small right-sided atelectasis	25
Fever	30
Moderate abdominal pain	17

Mortality

Three patients died in connection with **the treatment** (hospital mortality 3/49 treatments). One of our very first patients developed an abscess as a consequence of total splenic infarction, one patient with end-staged liver disease developed bronchopneumonia, which was resistant to antibiotics and a third patient developed pleural effusion, rebled and died. Another eight patients died within half a year, following the first treatment. Seven of them due to decompensated livers and one from variceal bleeding. All the patients dying within half a year had liver cirrhosis.

Late deaths

Three patients died in the period after four years from the embolization. One patient progressed in his liver disease and rebled. An attempt to stop this bleeding by mesocaval shunt was done. It can be argued whether this patients should have been operated on or not. Two patients developed malignancies. One patient died in pancreatic carcinoma, five years after the embolization. He had no rebleeding during those five years. Another patient died in nephrocarcinoma four and a half years after the embolization. With the exception of one small bleeding also this patient was bleeding free during the time interval.

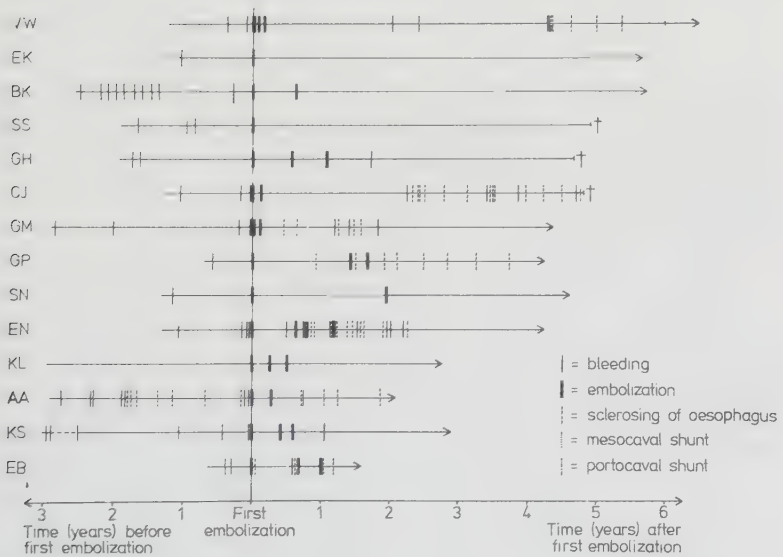


Fig I 2. Bleeding episodes and the different steps of treatment given to 14 patients with hypersplenism and portal hypertension.

Survival

Fourteen patients survived more than 4 years, or are still alive, six patients with portal or splenic venous thrombosis, eight patients with liver cirrhosis. Seven of the patients had no re-bleeding, seven rebled and had to be the object of further treatment. Fig. 1 2 indicates the bleeding episodes and the different steps of treatment given to these patients. We added periods from the first bleeding till the embolization treatment was finished for these 14 patients. They were shown to have 53 bleeding episodes during their 25 pretreatment years, i.e. an average of 2.12 bleeding episodes a year. After the treatment these patients lived altogether 45 years, and showed during that time 18 bleeding episodes, i.e. 0.4 bleeding episodes a year. Nine of the patients had additional therapy. Sclerotherapy of the esophageal varices was given to seven patients, and an emergency portocaval or mesocaval shunt was done in one patient respectively.

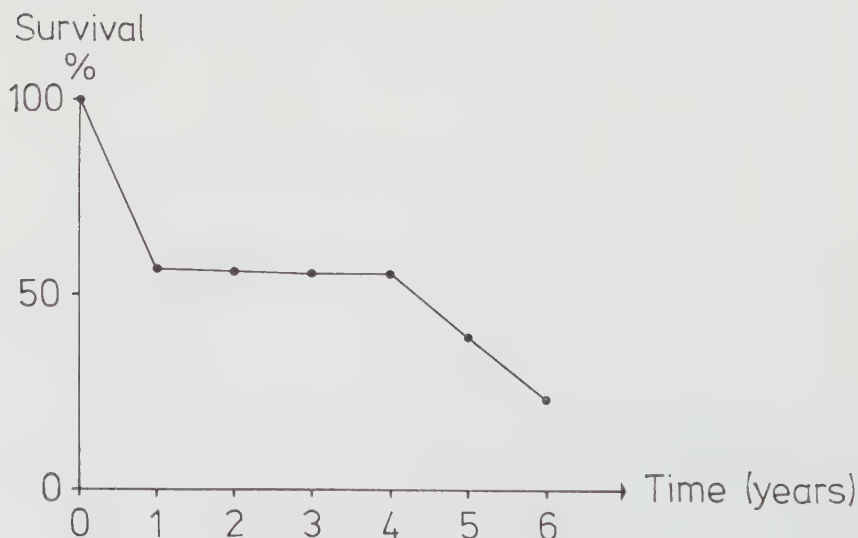


Fig I 3. The accumulated survival rate in 25 patients treated with splenic embolization.

Influence on peripheral blood count

The influence of the embolization on the peripheral blood elements is demonstrated in fig. I 4. The mean WBC count doubled during the first 24 hours, and remained at approximately that level during the subsequent fortnight.

The hemoglobin concentration did not differ from the subnormal pre-treatment level, during the first two weeks.

The mean platelet count rose significantly. It was back to normal values within one week and it was still rising two weeks after the treatment. In 14 of the patients, the platelet count was studied again after half a year - being within normal values (fig. I 5). Eleven patients were followed two years or more. A slight decline, in some cases to sub-normal value, was observed in these patients (fig. I 6).

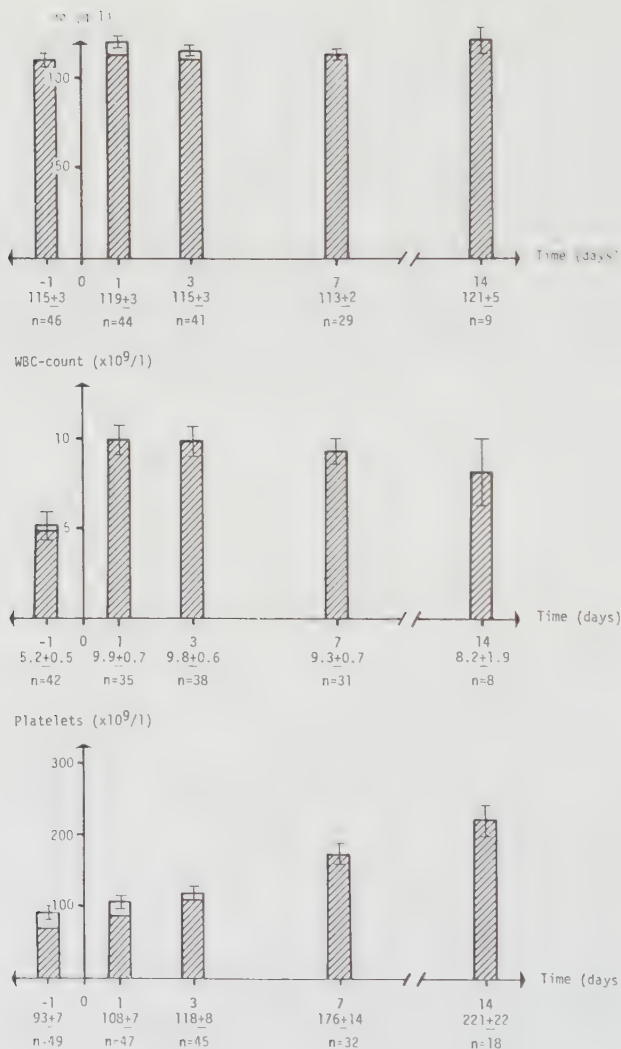


Fig I 4. Number of peripheral blood elements different time intervals after splenic embolization as compared to pre-treatment values. Filled parts indicate values from the first treatment in each patient (values given + SEM).

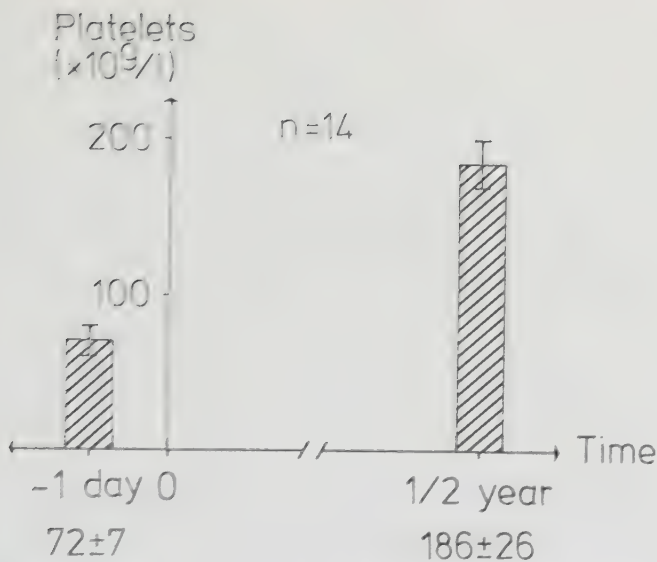


Fig I 5. Number of platelets in 14 patients half a year after splenic embolization as compared to pre-treatment values (values given $\pm$ SEM).

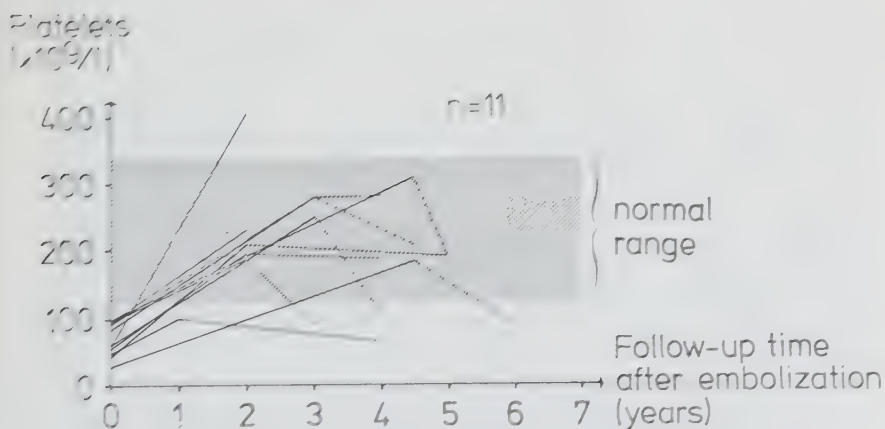


Fig I 6. Long-term follow-up of platelet counts in 11 patients after splenic embolization.

DISCUSSION

No curative, only symptomatic, treatment is possible when dealing with liver cirrhosis. The symptoms vary from one patient to another. One of the fairly often seen symptoms is hypersplenism, defined by Doan and Dameshek as splenomegaly combined with anemia and/or leukocytopenia and/or thrombocytopenia with hyper-activity of the corresponding bone marrow precursors, all normalized after splenectomy (14, 15). Splenectomy is well documented to have a beneficial effect on hypersplenism. It also reduces the amount of blood entering the portal circulation (54, 55). These two documented effects are supposed to lower the risk of rebleeding.

Splenectomy, with the aim to reduce hypersplenism and bleeding frequency, was performed in patients with portal hypertension as early as in the beginning of this century (2). Mayo reported in 1929 his results based on 144 splenectomies on patients with Banti's disease (32). The operative mortality at that time was slightly more than 10 %. During the following 15 years, several authors described beneficial effects of splenectomy; improvement of anemia, thrombocytopenia, sometimes hepatic function and reduction of gastrointestinal bleeding frequency (for review see Pemberton & Kiernan, 37). During the 40's and 50's several authors discussed the high bleeding frequency and disfavoured the method (33, 40, 42). The reports by Blakemore, Lord and Whipple in 1945 focused the interest on the portocaval anastomosis and its new possibilities (5, 52). Splenectomy was condemned, especially in extra-hepatic portal hypertension since it was suggested that it might destroy the last chance to perform a portosystemic shunt (4, 22). Later on, Hallenbeck and coworkers (23) and Hsu (25) compared the results of portosystemic shunts and splenectomy, not being able to describe any differences in long-term results. As a consequence of the reduced enthusiasm for portosystemic shunts, several surgeons have during the last decade advocated splenectomy in combination with other bleeding preventing methods; coronary vein ligation, transection of the esophagus, selective vagotomy or extensive paraesophageal devascularization (24, 26, 44, 48). After an era during which most of the interest was focused on esophageal bleeding varices and most of the treatments used concentrated on portosystemic shunts, it became obvious that the disease is complex and multi-symptomatic. We have been increasingly convinced that at present time treatment of portal hypertension and its complications, should be as little invasive as possible and as selective as possible depending on the symptoms (3).

One disadvantage with the total removal of the splenic tissue is the post-splenectomy sepsis described by King & Shumacker in 1952 (27) and later on confirmed by several investigators (20, 39, 43). This has led to a search for methods which reduce hypersplenism but conserve splenic function. Splenic artery ligation was shown to give good results on the peripheral blood count. Because of collateral development the effect was, however, not long lasting (57,

58). This problem also affects splenic artery occlusions by steal coils (51), Bucrylate^R (21) or balloon catheters (56). A more long lasting effect was obtained by Maddison in 1973, who used autologous blood clot (31) and later on by authors using minute Gelfoam particles (34, 46, 47, 53) producing partial splenic infarction and reduction of splenic parenchyma.

Table I C summarizes the literature from Maddison's work in 1973 to this paper. Apart from the work by Spigos et al, who in 1980 presented 41 cases, none of the papers have reported more than a few patients. Most of the writers reported an unacceptable high rate of complications and disfavoured the method. The reason for the high complication rate seems to be that the patients were over-treated resulting in total splenic necrosis and subsequent complications, often being fatal. It is of importance to stress the method of partial infarction and the obvious need for antibiotic cover. Some authors reported the splenic embolization as just a means to perform a safer splenectomy (8, 12, 29, 53).

The immediate effect of the splenic embolization is in good accordance to the experience in individual patients (6, 18, 31, 35, 45) or the experience in the series of Spigos et al (46, 47).

The increase in platelet count probably reflects the reduced trapping capacity of thrombocytes in the embolized spleen. In a few cases subnormal values of platelets 3-4 years after the initial treatment were observed together with a scintiscan showing a large spleen. This indicates that the embolic treatment must be repeated, even if more than 75-85% of splenic parenchyma is considered reduced at the initial embolizations. There are no long-term follow-up results reported for comparison.

The increased WBC-count perhaps more reflects activation of defense mechanisms against the dead splenic tissue than a decreased sequestration rate of white blood cells. Furthermore only a few patients exhibited subnormal pretreatment WBC-count.

The pretreatment hemoglobin concentration of these hypersplenic patients varied considerably mostly due to if the patient had been bleeding or not just prior to the embolization. The mean value, however, remained after the embolization subnormal although several patients followed for more than one year without serious bleeding had a normalized Hb.

The long-term follow-up showed a good effect of repeated partial splenic embolization also on recurrent bleeding as there was a significant reduction of bleeding episodes expressed as haemorrhages/years of observation.

All complications were seen as an immediate sequel to the embolization. The first noticed reaction only a couple of hours after the treatment was moderate abdominal pain after one third of the embo-

Table 1 C. Summary of the literature on splenic artery embolization

Author	Material of embolization	Percentage of embolized spleen	Indication	No. of pat.	Ab.	Complications	Mors
Maddison (1973)	autologous blood clots		bleeding from oes. varices	1	-	-	-
Bücheler et al. (1975)	fibrin foam	60-80 %	bleeding from oes. varices	2	-	hepatic coma 1	+
Goldstein et al. (1976)	gel foam + steel coils	100 %	hypersplenism	2	-	abscess 1 pain, pleural effusion 1	+ -
Witte et al. (1976)	gel foam balloon cath.	90 %	hypersplenism liver cirrhosis	3	-	abscess 1 abscess, pneum. 1 pneumonia 1	+ + +
Papadimitriou et al. (1976)	gel foam	80 %	hypersplenism liver cirrhosis	1	-	intest. paralysis, pain, pleural effus. 1	-
Castaneda-Zuniga (1977)	polyvinyl alcohol (Ivalon ^R)	100 %	thrombocytopenia ^x	3	-	septic shock 1 abscess 1 pleural effus. 1	+ + -
Conroy et al. (1978)	gel foam	100 %	hypersplenism ^x Hodgkin's dis.	1	-	-	-
Wheley et al. (1978)	steel coils balloon cath.	100 %	hypersplenism	3	-	abscess 1 splen. rupture 1	- -
Levy et al. (1979)	steel coils	100 %	hypersplenism ^x	1	-	-	-
Günther et al. (1980)	butyl-2-cyanoacrylate (Bucrylate ^R)	100 %	bleeding from oes. varices	1	-	necrosis of gastric mucosa 1	+
Spigos et al. (1980) [incl. Spigos (1977), Spigos (1979)]	gel foam	60-70 %	hypersplenism renal insuff. 35 thalassemia maj. 5 spl. v. thromb. 1	1	+	abscess 1 pancreatitis 1 pleural effus. 2 pneumonia 5	-
Vujic et al. (1981)	gel foam	50 %	hypersplenism liver failure	3	+	pneum. sepsis 1 abscess 1 pulm. emb. 1	+ + +
Gerlock et al. (1981)	gel foam	60-80 %	hypersplenism renal insuff.	6	+	-	-
Alwmark et al. (1982)	gel foam repeated treatments	30-50 %	hypersplenism portal hypertension	25	+	abscess 1 pneumonia 1 (rebleeding 1)	+ + +

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lizations. A non-narcotic analgesic drug for a few days was enough as treatment. No epidural anesthesia or intraarterial injections of chloroprocain (36, 47) was considered necessary. Fever, lasting for 2-5 days and often accompanied by small left-sided atelectasis and/or plural effusion was noticed the first posttreatment day in two thirds of the patients. More serious infectious complications were restricted to the first two patients in our series who developed splenic abscesses as a consequence of total infarction. A similar observation after extended or total infarction has been reported by many authors (51, 53, 56). The amount of dead tissue seemed too large leading to bacterial invasion despite antibiotic treatment. Later on in our series when the intended partial embolization of about 25-40% of the parenchyma was achieved, no abscesses were noticed. Spigos has reported the same experience in more than 35 uremic patients where partial splenic embolization under an adequate antibiotic cover was successfully performed (47). The systemic antibiotics are needed to prevent overgrowth of bacteria introduced from the skin (53) or portal blood (31, 49). The local antibiotics given as the soaked pieces of gelatin sponge and as an intraarterial dose after the procedure seemed to be a good adjuvant therapy (34, 46, 47).

Some reports indicate that patients with portal hypertension have an increased tendency to thrombotic disease (7, 10). Splenic vein thrombosis, seen in our four patients, could be related to the patient's underlying disease and the splenic embolization might be a triggering factor by way of stagnant flow and increased platelet counts. Non of the splenic vein thrombosis, however, proved to be fatal.

The hospital mortality in our series was 12%, a figure comparable to the mortality reported from splenectomies of Banti's disease in the 20's (32). Fabri reported in 1974, 13,5% postsplenectomy mortality in "secondary hypersplenism" (17). All of our patients, except one, had a history of bleeding oesophageal varices and many had severe liver disease (Child B and C) explaining the high mortality from liver insufficiency during the first six months after the treatment (Fig.I 3).

In our opinion the repeated partial splenic embolization is a good alternative to splenectomy in patients with hypersplenism secondary to portal hypertension. The bleeding episodes decline in frequency and the thrombocytopenia improves. The procedure is simple and if correctly performed gives no infectious complications but may give an immunologic functioning splenic remnant. We have found splenic embolization to be a good means of selective symptomatic treatment of one of the complications to portal hypertension.

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TREATMENT OF HYPERSPLENISM - AN EXPERIMENTAL STUDY IN THE RAT

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ABSTRACT

Methylcellulose injected intraperitoneally into rats proved to give a splenomegaly combined with anaemia and thrombocytopenia. The WBC-count did not change during the treatment. The splenic parenchyma of the hypersplenic rats was then reduced by resection or splenic artery ligation to different extent. The peripheral blood cell count was normalized after one third resection whereas more than two thirds reduction of the splenic parenchyma caused infective complications in many rats. The "secondary hypersplenism" in rat was thus possible to treat by a partial reduction of the splenic parenchyma avoiding the, with new immunologic knowledge, much undesired total splenectomy.

Key words: Hypersplenism, Methylcellulose, Peripheral blood cell count.

INTRODUCTION

Already in 1883 Banti described anaemia combined with splenomegaly (3). Throughout the years many investigators have found that a hyperfunctioning spleen may reduce the platelet count, induce anaemia and sometimes a low white blood cell count (WBC). After work by Doan 1949 and Dameshek 1955 the term hypersplenism is now well-established (7, 9). The usual treatment has been splenectomy but after reports that this operation is followed by an increased risk for fatal sepsis not only in childhood but also in adults (12, 21), alternatives to splenectomy have been searched for. Ischaemic therapy such as splenic artery ligation, arterial balloon occlusion or arterial embolization have been advocated as means to reduce functional splenic mass (23, 26, 30). We have recently used pieces of gelatin sponge for embolization of the splenic parenchyma as an alternative to splenectomy in patients with portal hypertension and hypersplenism (17). Promising results have been recorded from this method but an optimal degree of reduction in order to obtain a normalization of peripheral cytopenia has not yet been established.

In the present study, in an attempt to simulate the clinical situation, the effect of partial splenectomy and peripheral splenic artery ligation on peripheral blood cell counts was investigated in rats with hypersplenism induced by methylcellulose as described by Palmer et al. 1953 (18).

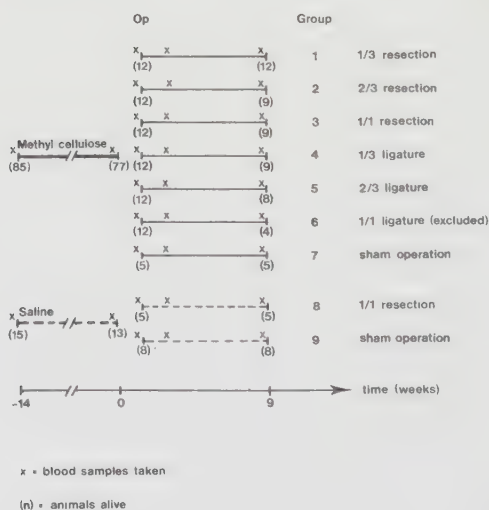


Fig. II 1. Different treatment of hypersplenism after 14 weeks of methylcellulose administration to rats.

MATERIAL AND METHODS

Eighty-five male Sprague-Dawley rats weighing about 250 g were given 2 ml of a 2.5% methylcellulose solution intraperitoneally twice weekly for 14 weeks (Methocel A4, Dow Chemicals, 2160 Stade Brunnshausen, West Germany). A further 15 rats were put through an identical procedure but given 2 ml saline instead. All animals were kept in cages, 5 rats in each, under ordinary laboratory conditions with food (pellets) and water ad lib. After 14 weeks of treatment the rats were divided into 9 groups according to Fig. II 1.

The following types of operations were performed in the different groups.

Group 1. Through a midline abdominal incision the spleen was mobilized. After ligation of the corresponding vessels using No. 6/0 silk, the approximated lower third of the spleen was resected. To achieve haemostasis after the resection performed, a catgut ligature was applied around the splenic remnant close to the resected surface.

Group 2. The approximated lower two thirds of the spleen were resected by the same procedure.

Group 3. Total splenectomy was performed.

Group 4. Following mobilization of the spleen, the peripheral vessels estimated to supply the lower third of the spleen were ligated and divided close to the spleen. The spleen was left in situ.

Group 5. Using the procedure described for group 4 the vessels estimated to supply the lower two thirds of the spleen were ligated and divided.

Group 6. Using the procedure described for group 4 all the splenic vessels were ligated and divided.

Group 7. A splenic mobilization (sham-operation) was performed in these rats.

Group 8. These animals were given i.p. injections of saline twice weekly during 14 weeks and were then splenectomized.

Group 9. Saline injections were given as in group 8. A sham-operation was performed.

All animals were operated on under light ether anaesthesia and under sterile conditions.

Ten days postoperatively to maintain the hypersplenic stimulus, i.p. injections of methylcellulose twice weekly were again instituted in groups 1-7 and saline was given to groups 8 and 9. The treatment was continued for 9 weeks. The weights of the animals were determined and blood samples drawn from the tail vein. Haemoglobin concentration, white blood cell count and platelet count were determined as shown in Fig. II 1. Samples were taken before administration of methylcellulose, 14 weeks later, 1 week, 3



Fig. II 2. Difference in size between the spleen from a rat given methylcellulose for 14 weeks (left) and the spleen from a control rat (right).

weeks and 9 weeks postop. i.e. immediately before killing the animals. The weights of the removed part of the spleen and the remaining splenic tissue at autopsy were determined.

Hemoglobin (Hb) was determined photometrically according to Kampen and Zijlstra (1961). Platelet counts and white blood cells were determined in a Bürkner chamber, using a phase contrast microscope.

Statistical methods

Student's t -test for unpaired data was used to evaluate methylcellulose treatment and to compare effects of different operations. Student's t -test for paired data was used to evaluate the treatment within each group.

RESULTS

Initial methylcellulose treatment

Of the initial 85 rats treated with methylcellulose, 5 died before 14 weeks and in 3 rats blood sampling was unsuccessful. All 77 studied rats showed a marked splenomegaly (Fig. II 2) with significantly lowered haemoglobin concentration values and platelet count compared with 13 control rats given only saline i.p. (Table II A). White blood cell count did not change during the treatment. There was no significant difference between the weight of the rats given methylcellulose and that of the control rats (Table II A).

Table II A. Changes in body weight and peripheral blood elements during 14 weeks of methylcellulose administration to rats^{a)}.

	Weight of rats	Hemoglobin concentration (g/l)	WBC count ($\times 10^9/l$)	Platelet count ($\times 10^9/l$)
Hypersplenic rats n = 77				
Before	239 $\pm$ 2.3	152.9 $\pm$ 0.74	12.8 $\pm$ 0.42	896 $\pm$ 18.0
After	444 $\pm$ 4.6	^{s)} 144.6 $\pm$ 1.97	12.7 $\pm$ 0.43	^{s)} 642 $\pm$ 12.6
Control rats n = 13				
Before	247 $\pm$ 5.6	150.2 $\pm$ 2.69	13.2 $\pm$ 1.02	788 $\pm$ 41.0
After	463 $\pm$ 9.1	^{s)} 166.6 $\pm$ 2.06	11.0 $\pm$ 0.95	^{s)} 824 $\pm$ 25.7

^{a)} Values are given as mean $\pm$ standard error of the mean.

^{s)} The difference between the animals given methylcellulose and saline are significant ($p < 0.001$).

Table II B. Changes in body weight and peripheral blood elements after various reduction of splenic parenchyma.

	Weight of rats	Haemoglobin concentration (g/l)	WBC count ($\times 10^9/l$)	Platelet count ($\times 10^9/l$)
1 n = 12 (1/3 resection)	465 $\pm$ 8.8 473 $\pm$ 12.4 n.s.	156 $\pm$ 14.3 159 $\pm$ 8.9 n.s.	12.0 $\pm$ 0.9 28.8 $\pm$ 3.9 $p < 0.005$	618 $\pm$ 10.7 901 $\pm$ 15.1 $p < 0.005$
2 n = 9 (2/3 resection)	436 $\pm$ 24.0 456 $\pm$ 12.4 n.s.	145 $\pm$ 13.0 146 $\pm$ 16.2 n.s.	11.9 $\pm$ 1.0 27.1 $\pm$ 3.8 $p < 0.005$	637 $\pm$ 37.3 1052 $\pm$ 57.0 $p < 0.001$
3 n = 9 (1/1 resection)	463 $\pm$ 10.4 461 $\pm$ 13.2 n.s.	152 $\pm$ 6.0 155 $\pm$ 11.3 n.s.	12.5 $\pm$ 1.9 31.6 $\pm$ 4.4 $p < 0.005$	565 $\pm$ 48.9 822 $\pm$ 36.4 $p < 0.001$
4 n = 9 (1/3 ligation)	435 $\pm$ 11.9 427 $\pm$ 28.0 n.s.	131 $\pm$ 16.1 142 $\pm$ 13.0 $p < 0.05$	11.9 $\pm$ 1.7 26.6 $\pm$ 5.5 n.s.	692 $\pm$ 34.9 888 $\pm$ 57.1 $p < 0.01$
5 n = 8 (2/3 ligation)	417 $\pm$ 13.2 425 $\pm$ 24.0 n.s.	130 $\pm$ 12.6 137 $\pm$ 22.1 n.s.	15.5 $\pm$ 1.7 39.0 $\pm$ 7.0 $p < 0.02$	685 $\pm$ 25.3 1026 $\pm$ 39.2 $p < 0.001$
7 n = 5 (MC + sham)	409 $\pm$ 2213 432 $\pm$ 28.6 n.s.	142 $\pm$ 9.2 146 $\pm$ 8.4 n.s.	7.3 $\pm$ 1.4 11.8 $\pm$ 3.6 n.s.	600 $\pm$ 37.4 717 $\pm$ 27.8 n.s.
8 n = 5 (NaCl + 1/1 res.)	481 $\pm$ 18.8 485 $\pm$ 21.0 n.s.	171 $\pm$ 3.6 166 $\pm$ 4.0 n.s.	13.1 $\pm$ 3.1 17.1 $\pm$ 0.8 n.s.	848 $\pm$ 15.3 930 $\pm$ 53.3 n.s.
9 n = 8 (NaCl + sham)	453 $\pm$ 7.9 445 $\pm$ 12.0 n.s.	163 $\pm$ 7.6 162 $\pm$ 10.4 n.s.	9.8 $\pm$ 1.0 13.7 $\pm$ 1.4 n.s.	855 $\pm$ 77.7 761 $\pm$ 50.7 n.s.

Different operative methods

In all groups, except one, a few rats died before the final blood samples were obtained. All these rats as well as all animals in the group of "total ligature" (8 of 12 rats died before 9 weeks) were excluded from the final calculations. Autopsy performed on every killed rat showed in a few animals intraabdominal infections, that were more frequent if the vessels were ligated to a large extent. Cultures revealed growth of *E. coli* and *Streptococcus faecalis*. A couple of rats had an ulcer of the stomach and in a few rats no explanation to their death was found.

During the nine weeks after various operations all rats gained or lost in body weight only a few grams, not showing any significant differences (Table II B).

The weights of spleens previously reduced by one third differed significantly from those reduced by two thirds. However, no difference in splenic weight was noted in rats subjected to splenic resection when compared with artery ligation to the corresponding extent. From the same figure is also seen that the weight of the remaining spleen after one third resection and one third ligature was significantly less than that of the sham-operated methylcellulose treated rats (Fig. II 3).

The effects of the different operative methods on peripheral blood elements nine weeks postoperatively are presented in Table II B. A slight increase in hemoglobin concentration was noted in all animals subjected to spleen reduction; however, this was statistically significant only in group 4 ($p < 0.05$). Nine weeks postoperatively a significant increase in WBC-count was seen in spleen-resected rats ($p < 0.005$). In animals subjected to splenic ligature (group 4 and 5) only two thirds reduction significantly ($p < 0.02$) increased WBC-count. As also seen in Table II B. Animals in group 1-5 showed a significant increase in platelet count regardless of the surgical procedure performed. Splenic operations were in all rats followed by a prompt increase in platelet count. The most rapid response was obtained after splenic resection but the results after three and nine weeks respectively did not differ in spleen-resected compared with spleen-ligated animals (Fig. II 4).

DISCUSSION

The spleen acts like a filter in the blood stream collecting and removing defective red blood cells, damaged white blood cells and old platelets. If, for some underlying reason (such as spherocytosis, mononucleosis, myeloid metaplasia) the splenic work load is increased, the spleen will grow in size and function. The same is seen in the cirrhotic patient with an often hyperdynamic portal blood flow and a so-called congestive splenomegaly, the cause of which is much debated (25, 26). The result will be the state of hypersplenism - defined among others by Jandl & Aster (15) as splenomegaly, a reduction in one or more cellular elements of blood with hyperplasia of their respective marrow precursors, and correction of the blood cytopenia by splenectomy.

Spleen weight
g

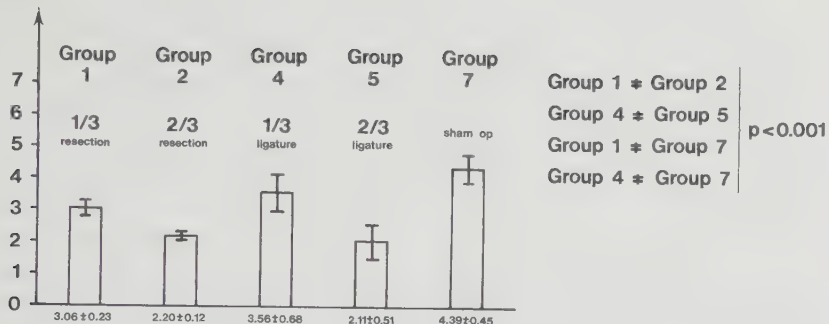
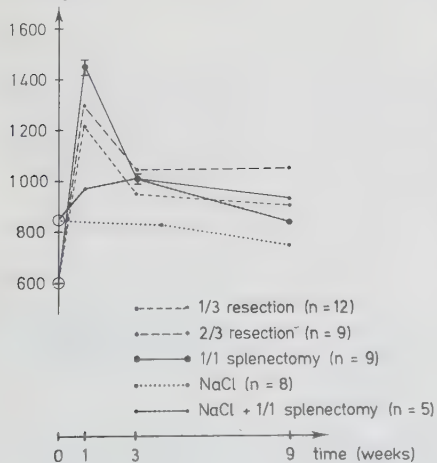


Fig. II 3. Weight of remaining splenic tissue nine weeks after different operations.

Platelets
($\times 10^9/l$)



Platelets
($\times 10^9/l$)

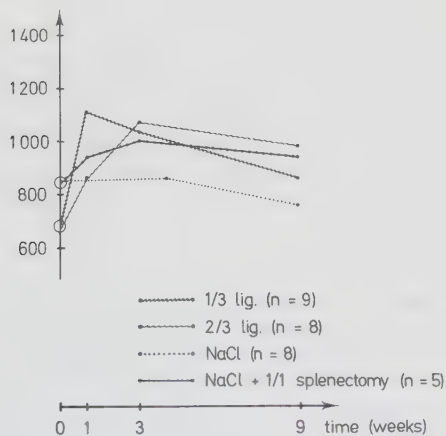


Fig. II 4. Platelet count after various amounts of splenic reduction.

Removal of the spleen has today a low immediate mortality rate (7). However, complications such as haemorrhage and infections are often reported in children and in patients with large spleens, severe thrombocytopenia, liver cirrhosis (6, 22). These operative complications together with the increased risks of serious postsplenectomy infections prompted other methods of treating hypersplenism, viz. splenic artery ligation (26, 27) and embolization of the splenic parenchyma (5, 19, 23). Ligation or transcatheter occlusion of the splenic artery may effectively improve the hypersplenic state although this improvement is often transient due to development of collaterals (13, 24, 26). Embolization of the spleen via the splenic artery in a human was first performed by Maddison (1973) using autologous blood clots (16). This material as well as Gelfoam^R will give a peripheral infarction without development of collaterals and with long-standing effect.

A major complication has been abscess formation following total splenic infarction (13, 24, 29). In our series of more than 20 treated patients, abscesses developed in two, both with an infarction of more than 75% of the splenic parenchyma (17). This gave rise to the question of the degree of optimal resection necessary to avoid an abscess development while still achieving normalization of the peripheral blood elements.

This was studied in an experimental rat model using methylcellulose injected intraperitoneally. The overload hypersplenism hereby obtained, although differently induced, may to some extent reflect the changes following congestive hypersplenism in cirrhotic patients (1, 4, 8, 11, 26, 31). The methylcellulose treatment was well tolerated by the animals as shown by a body weight gain not differing from that of saline treated control rats. A fivefold increase in splenic weight, anaemia and thrombocytopenia was noted whereas the WBC-count did not change.

The rats with induced hypersplenism were treated by splenic resection or peripheral splenic artery ligation, the latter method producing an ischaemic spleen remnant thus simulating embolization of the splenic parenchyma. The weights of remaining splenic tissue recorded at autopsy indicated that reduction by ligature gave a functional splenic mass which did not differ in size from that obtained by corresponding resection. Regeneration at nine weeks was higher after two thirds reduction compared with after one third reduction; however, significant regeneration occurred in both groups.

Haemolytic anaemia with a high rate of cell destruction in the spleen is known to develop in rats given methylcellulose, as in this study (4, 18). The slow increase in haemoglobin concentration here noted after reduction of the spleen, statistically significant in one group, may conceivably reflect the normalization of the splenic function.

The WBC-count did not change during the methylcellulose administration in contrast to the findings of Palmer (18). An increased sequestration rate has been shown to be the reason for the hypersplenic leukocytopenia (20). The result obtained after splenic reduction was in our study a significant increase in WBC-count after spleen resection or two thirds ligation.

tion. This probably rather reflects an infectious state than a leukocytosis depending on the spleen operation in se, as much as the animals never had subnormal values of WBC-count.

The hypersplenic thrombocytopenia is considered mostly to be due to a splenic trapping of platelets as evidenced among others, by Jacob and Aster (2, 14) who found a decrease in platelet count across the enlarged spleen, and Jandl & Aster (15) who found accumulation of radioactivity over the enlarged spleen after injection of ^{51}Cr -labelled platelets. The various reducing operations performed in this study were all followed by a rapid increase in platelet count. The response after resection and total splenectomy did not differ much whereas the increase in platelet count after ligature was somewhat slower, conceivably due to a more gradual reduction of the splenic pool of platelets after ligature. The results after 9 weeks, however, did not differ between the various groups.

To sum up, this study shows that when more than 65% of the splenic vessels were ligated in hypersplenic rats many of the animals developed lethal infections. On the other hand reduction of one third of the splenic parenchyma was comparable to two thirds reduction regarding normalization of depressed peripheral blood counts occurring as a consequence of hypersplenism. A total splenectomy was not followed by better values of the peripheral blood elements than was a partial reduction of the spleen. Even if the function of remaining splenic tissue was not determined, it is reasonable to suppose this condition to be superior to the asplenic state.

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INCREASED SUSCEPTIBILITY OF HYPERSPLENIC RATS
TO INFECTION WITH PNEUMOCOCCI

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Alwmark, A., Christensen, P., Gullstrand, P. & Schalén, C. Increased susceptibility of hypersplenic rats to infection with pneumococci. Acta path. microbiol. immunol. scand. Sect. B, - - -

Hypersplenism was induced in rats by intra-peritoneal injections of methylcellulose. These rats developed an outsize spleen and had significantly depressed values for hemoglobin concentration, hematocrit and platelet count. The rats given methylcellulose were also found to be more susceptible to a challenge with a standardized intravenous injection of *Streptococcus pneumoniae* and had a significantly higher mortality rate than the control rats.

Key words: Hypersplenism; pneumococcal infection; rat.

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Overwhelming infection occurs by increased frequency in clinical conditions associated with absence of splenic function and is associated with a significant mortality (6, 12). Encapsulated bacteria account for more than 75 % of the septic events in splenectomized individuals, and *Streptococcus pneumoniae* is the most prevalent (8). Experimental studies with laboratory animals, in particular the splenectomized rat model, have demonstrated the importance of the spleen in effecting clearance of pneumococci upon intravenous injection (3, 7, 9, 13, 14).

The term "hypersplenism" has been used to denote a syndrome characterized by increased destruction of normal blood cells by a malfunctioning spleen and/or by possible splenic inhibition of the bone marrow (4, 5). As a result, anemia, neutropenia, thrombocytopenia - or any combination of the three - develops. In spite of these well known characteristics of hypersplenism, little attention has been paid to the susceptibility of such individuals to infections.

In 1953, *Palmer et al.* (11) described a model for induction of hypersplenism in rats, using repeated injections of methylcellulose. The present paper concerns the susceptibility of such animals to pneumococcal challenge. We have recently described the use of frozen inocula of pneumococci in splenectomized rats, which allows a more exact titration of the number of bacteria lethal to experimental animals (1). This paper concerns the susceptibility of hypersplenic rats to pneumococcal infection, taking advantage of our previously described method.

MATERIALS AND METHODS

Animals

One hundred male Sprague-Dawley rats initially weighing about 225 g were divided into two groups: 80 of them were given 2.0 ml methylcellulose of 400 centipoise with the concentration of 2.5 g per 100 ml water (Methocel A4, Dow Chemicals, 2160 Stade-Brunshausen, West Germany) and 20 animals were injected with 2.0 ml saline intraperitoneally twice weekly for 12 weeks (11).

Challenge of the Animals with Pneumococci

Pneumococci type 1 were prepared and stored at -80°C as previously described (1). After the 12 weeks of pre-treatment the rats were divided into 5 groups as shown in Table III A. The pneumococci were thawed and immediately injected intravenously, into the hind limb or the penile vein. The rats were then observed every sixth hour for the following 2 weeks. All animals dying were autopsied and a portion of the heart blood was inoculated overnight at 37°C on haematin agar plates. Two weeks after pneumococcal challenge, a heart puncture was made in each of the surviving rats and the blood obtained was cultured.

TABLE III A. The Susceptibility of Hypersplenic Rats to Infection with Pneumococci

Pretreatment of animals	N. of animals	CFU of pneumococci given intravenously	N. of animals killed	Survival time (hours) of animals killed mean (range)
Methylcellulose	20	4×10^3	0	-
Methylcellulose	20	4×10^4	2	116 (72-160)
Methylcellulose	20	4×10^5	7 ^a	90 (43-134)
Methylcellulose	20	4×10^6	8 ^b	124 (68-207)
Saline	20	4×10^6	0	-

a) The difference between this group and the group given saline was significant ($p = 0.003$).

b) The difference between this group and the group given saline was significant ($p = 0.001$).

Blood Cell Counts

Blood was obtained from the rats before the injections of methylcellulose and saline were started, and immediately before challenging with pneumococci, for determination of hemoglobin, blood-erythrocyte volume fraction and white blood cell (WBC) and platelet counts.

Statistical Analysis

The Chi-Square test (exact method) and Student's t -test for paired observations were used.

RESULTS

All 20 rats given saline intraperitoneally survived the intravenous challenge with 4×10^6 CFU (colony forming units) of pneumococci. In contrast, 8 of 20 rats given methylcellulose died within 9 days when this dose of bacteria was injected ($p = 0.001$; Table III A). When the dose of bacteria was reduced to 4×10^5 CFU, 7 of 20 methylcellulose-given rats died ($p = 0.003$). However, challenge of such animals with 4×10^4 bacteria did not result in more deaths than in the case of the controls.

Heart blood taken from those animals that died in these experiments all grew pneumococci. The 83 surviving animals were subjected to heart puncture 2 weeks after the pneumococcal challenge: the blood of one of the methylcellulose-treated rats contained pneumococci belonging to capsular type 1, *viz.* the strain injected. The blood from the remaining 82 animals was culture-negative.

The weights of the spleens taken at autopsy ranged between 4.85 and 9.60 g (mean 8.05 g) in the rats receiving methylcellulose, in contrast to 0.87 to 1.05 (mean 0.96) in animals given saline. Hemoglobin concentration, hematocrit, WBC count, platelet count and body weight of the rats before and

TABLE III B. Changes in Peripheral Blood Elements in Methylcellulose-Treated Rats^{a)}

	Weight (g)	Hemoglobin (g/l)	Hematocrit	White blood cell count	Platelet count
Before methylcellulose administration (n = 80)	227±5.2 (181-283)	147.4±1.19 (114-178)	43.6±0.51 (38-57)	11.9±0.45 (3.6-9.6)	891±15.9 (556-1228)
After methylcellulose administration	425±6.6 (270-511)	^b 118.4±1.29 (98-150)	^b 34.5±0.41 (28-45)	10.5±0.55 (4.4-20.8)	^b 529±12.3 (278-952)
Before saline administration (n = 20)	225±5.3 (181-268)	142.5±2.02 (126-158)	42.6±0.37 (40-46)	10.67±0.69 (4.8-15.8)	903±40.2 (392-1220)
After saline administration	417±6.1 (345-476)	^b 162.0±2.33 (145-184)	^b 46.3±0.62 (42-52)	11.0±0.74 (6.4-18.4)	^b 896±39.5 (428-1132)

a) Values are given as mean ± standard error of the mean, the values in parentheses denoting the range.

b) The differences between the animals given methylcellulose vs. saline are significant ($p < 0.001$).

after 12 weeks of methylcellulose administration are given in Table III B. There was a significant decrease in hemoglobin concentration, hematocrit and platelet count, but not in the WBC count or body weight in the rats given methylcellulose compared with the control rats. The WBC count of the surviving methylcellulose-treated rats did not differ from the values of those that died.

DISCUSSION

Rats made hypersplenic by injections of methylcellulose were more susceptible to pneumococci than were normal rats. While 4×10^6 pneumococci had no effect on the normal rats, 4×10^5 CFU killed 7 or 20 hypersplenic animals. It is worth noting, however, that the amount of pneumococci required to kill the hypersplenic rats, *viz.* 4×10^5 CFU, was about 1000 times greater than the lethal dose found in previous works for splenectomized animals. In this previous study, we employed the same batch of pneumococci as used in the present work and found 200 CFU to be lethal for all of 8 splenectomized rats challenged with this dose (1). Furthermore, the survival times after challenge in the killed rats were 34 to 132 hours (mean 36 hours) in the splenectomized group (1), as compared with 43 to 134 hours (mean 90 hours) of the methylcellulose-treated rats receiving 4×10^5 bacteria in the present investigation. These findings, taken together, clearly demonstrate that the hypersplenic rats succumbed to a more prolonged pneumococcal septicemia than was the case with the splenectomized animals. Thus, resistance of hypersplenic rats to pneumococci was impaired, compared with that of normal animals, though not to such a degree as seen in splenectomized rats.

The finding that 8 hypersplenic rats were killed, while 12 survived injection of 4×10^6 pneumococci, could not be explained by differences in the numbers of white blood cells. It remains to be seen if intrastrain diffe-

rences in the immune defense system are in some way contributory to the varying susceptibility recorded here. Several explanations might be proposed to explain the exact mechanism by which hypersplenism rendered the rats more susceptible to pneumococcal infection. Theoretically, the macrophages of the spleen could have been blocked by the injections of methylcellulose. Furthermore, impaired production of tuftsin should be considered, although little is known about this substance in hypersplenism. Tuftsin binds to polymorphonuclear neutrophils and stimulates their phagocytic effect (2). The physiological significance of tuftsin has been demonstrated in splenectomized patients and in individuals with a congenital tuftsin abnormality, in whom low serum levels of tuftsin coincide with a high incidence of infections (10).

In conclusion, hypersplenic rats were found to be more susceptible to pneumococcal challenge than normal rats. The possibility that hypersplenic patients might have impaired resistance to bacterial infections should therefore also be considered.

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INCREASED SUSCEPTIBILITY TO PNEUMOCOCCI AFTER LIGATION OF THE SPLENIC
ARTERY IN EXPERIMENTAL HYPERSPLENISM

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Key words. Experimental hypersplenism, Splenic artery ligation,
Pneumococci infection

ABSTRACT

Twentytwo male Sprague-Dawley rats were divided into two groups, of which group I received methyl-cellulose and group II saline intra-peritoneally for 12 weeks. After ligation of the splenic artery of the animals in group I and sham-operation of the rats in group II, the injections were continued for further 9 weeks. At the operation, the group I animals all showed signs of hypersplenism with anemia, and leuko- and thrombocytopenia. The platelet counts normalized after the operation, a marked leukocytosis developed and the anemia was further aggravated. At the end of the study, the animals were challenged with 4×10^6 colony forming units of pneumococci type 1, resulting in deaths of 11 of 12 animals in group I, in contrast to survival of all 10 rats in group II ($p = 0.000017$).

INTRODUCTION

Overwhelming infection after splenectomy is a serious threat, particularly in infants and young children with blood dyscrasias [11, 17]. Despite of this risk, the treatment of choice for hypersplenism, characterized by increased pooling and hypersequestering of normal blood cells [9, 13], has during the last decades been splenectomy. Recently, attempts have been made to reduce hypersplenism with alternative and less invasive techniques than total removal of the organ. It was thought that partial reduction of the splenic parenchyma would eliminate the hypersplenism without causing increased susceptibility to infection. Thus, partial splenectomy [5], partial dearterialization [18], and ligation [19] or embolization [4] of the splenic artery has been tried.

In normal rats, splenic artery ligation can be performed with a very low complication rate [6, 19, 21]. We reported in a previous study that hypersplenism induced by methyl-cellulose [16] in rats could be treated by partial resection or ligation of selected vessels to the spleen [2]. However, if all vessels entering the organ were ligated peripherally, abdominal abscesses developed in two thirds of the animals, leading to their deaths. These findings diverged from a report of Witte et al. [20] who were able to ligate the splenic artery in hypersplenic rats without any reported infectious complications.

Encapsulated bacteria, above all pneumococci, account for the majority of the septic events in splenectomized individuals [14]. The increased susceptibility to pneumococci after splenectomy is well demonstrated in laboratory animals, e.g. the rat [1, 12, 15]. Contrary to what may be expected, rats made hypersplenic by methyl-cellulose injections also reveal an increased susceptibility to pneumococci, although higher doses of bacteria are required to obtain a lethal septicemia than in splenectomized animals [3]. The present report concerns the effect of splenic artery ligation on the susceptibility of experimentally hypersplenic rats to challenge with pneumococci.

MATERIALS AND METHODS

Animals

Twentytwo male Sprague-Dawley rats, initially weighing about 240 g, were divided into two groups; 12 of them (group I) were given 2.0 ml methyl-cellulose of 400 centipoise with the concentration of 2.5 g per 100 ml water (Methocel, Dow Chemicals, 2160 Stade-Brunshausen, West Germany), and 10 animals (group II) were injected with 2.0 ml saline intraperitoneally twice weekly for 12 weeks [16]. After the operations (see later) the injections were restarted and continued for another 9 weeks.

The rats were kept under ordinary laboratory conditions, given pellets and water ad libitum.

Operations

The animals in group I were subjected to splenic artery ligation, using Zeiss OPMI 6-S operative microscope. Via a midline incision, the splenic artery was separated from the splenic vein and ligated just proximal to the branching of the upper splenic pool artery using Silk 6/0. The spleen was not mobilized.

The animals in group II were subjected to sham-operation, in which through a midline incision the splenic artery was dissected but not ligated. The operations were performed under ether anesthesia.

Challenge of the Animals with Pneumococci

Pneumococci type 1 were prepared and stored at -80°C as previously described [1]. After the final series of methyl-cellulose or saline injections, 4×10^6 colony forming units were injected intravenously, into the hind limb or the penile vein. The rats were then observed every sixth hour for the following two weeks. All killed animals were autopsied and a portion of the heart blood inoculated overnight at 37°C on haematin agar plates.

Blood Cell Counts

Blood was taken for determination of hemoglobin concentration, blood-erythrocyte volume fraction (hematocrit), white blood cell (WBC) and platelet counts just before starting the first series of injections, immediately before operation and one, three and nine weeks postoperatively, the last determination just prior to the pneumococcal challenge.

Statistical Analysis

Student's t-test was used for comparison of the means of the various values for the two groups, and Chi-Square test (exact method) to evaluate the outcome of pneumococcal challenge.

RESULTS

Hematological Findings

The two groups of animals, given methyl-cellulose and saline, were comparable before the injections were started, showing similar weights (240 ± 2.4 g (SEM) and 240 ± 5.0 g, respectively), hemoglobin concentrations, hematocrit, WBC and platelet counts (Table A). After 12 weeks of injections, immediately before operation, the animals in group I (methyl-cellulose treated) showed significantly decreased hemoglobin concentration, hematocrit, WBC and platelet counts, as compared to group II (given saline). One week after ligation of the splenic artery, the WBC and platelet counts were significantly higher in group I, followed by a normalization of the platelet counts, but a further increase in WBC at 3 and 9 weeks after operation. On the other hand, the hematocrit and hemoglobin values did not increase in group I. In contrast, the hemoglobin values were significantly lower 9 weeks after operation, as compared to the values immediately prior to operation ($p < 0.005$ (Table A)).

At the end of the study, the methyl-cellulose treated and splenic artery ligation, and sham-operated rats weighed significantly less than the animals given saline (386 ± 17.8 and 445 ± 12.0 g, respectively; $p < 0.05$).

Table A. Values of peripheral blood elements in splenic artery-ligated rats compared to sham-operated control rats.^a

Time of investigation	Hemoglobin (g/l)	Hematocrit	White blood count ^g (10 ⁹ /l)	Platelet count ^g (10 ⁹ /l)
<u>Before start of injections^b</u>				
group I	151 ± 1.1	44 ± 0.2	10.7 ± 0.60	918 ± 26.4
group II	148 ± 0.5	43 ± 0.5	12.8 ± 1.01	802 ± 38.0
<u>Preoperatively, after 12 weeks injections</u>				
group I	108 ± 2.6*	33 ± 0.9**	9.8 ± 0.64	338 ± 16.2*
group II	163 ± 2.0	44 ± 0.8	10.4 ± 0.95	820 ± 31.8
<u>One week postoperatively^c</u>				
group I	107 ± 5.7*	33 ± 1.6*	34.0 ± 3.70*	1613 ± 133.7*
group II	160 ± 7.0	48 ± 0.6	9.8 ± 1.80	860 ± 77.0
<u>Three weeks postoperatively</u>				
group I	101 ± 2.9*	34 ± 0.8	64.0 ± 11.6**	921 ± 50.2
group II	165 ± 8.8	49 ± 0.4	13.9 ± 5.5	838 ± 52.1
<u>Nine weeks postoperatively</u>				
group I	93 ± 3.2*	30 ± 0.7*	79.0 ± 11.5*	993 ± 45.1
group II	161 ± 9.4	49 ± 0.5	12.8 ± 0.9	812 ± 55.8

^a The values are given as means ± standard error of the mean

^b Group I was given methyl-cellulose and group II saline intraperitoneally

^c Splenic artery ligation was performed in group I and sham-operation in group II

* Group I differs significantly from group II ($p < 0.001$) ** Group I differs significantly from group II ($p < 0.005$).

Effect of Pneumococcal Challenge

The effect of administrating 4×10^6 colony forming units of pneumococci is shown in Table B. While only 1 of 12 animals subjected to splenic artery ligation survived, none of the 10 controls in group II were killed ($p = 0.000017$). All animals succumbing, died within 30 hours (median 17 h) after pneumococcal challenge. Heart blood from all killed rats grew pneumococci, while blood taken from all surviving rats was culture negative.

Table B. Survival of splenic artery-ligated hypersplenic rats compared with sham-operated rats after challenge with living pneumococci.*

Pretreatment of animals	Number of animals tested	Number of animals killed
Group I: methyl-cellulose for 12 weeks, splenic artery ligation and 9 further weeks injections	12	11
Group II: saline for 12 weeks, sham-operation and 9 further weeks injections	10	0

* Each animal was given 4×10^6 colony forming units of pneumococci type 1.

DISCUSSION

The present study verified the results of Witte et al. [20] that platelet count normalizes after splenic artery ligation in the rat with induced hypersplenism, while the leukocyte count gradually rises to a highly elevated level. Furthermore, it was found that the anemia, already present in the hypersplenic animals, was aggravated. Conceivable,

the concurrent leukocytosis and the **anemia** could well be explained by the splenic abscess formation, observed following ligation of the splenic artery. It is also to be pointed out, however, that the infusion of methyl-cellulose was continued after the splenic artery ligation. Thus, further studies are required to elucidate the role of methyl-cellulose in the aggravation of the anemia.

Nine weeks after ligation of the splenic artery, the hypersplenic rats were highly susceptible to pneumococci: 11 of 12 animals died within 30 hours after challenge whereas all controls survived (Table B). We have previously reported the effect of the same dose of pneumococci, 4×10^6 colony forming units, taken from the same batch of frozen bacteria, on methyl-cellulose treated rats which by all hematological data were comparable to the hypersplenic rats in the present investigation at the time of operation [3]. In this previous work, 8 of 20 rats given methyl-cellulose, died between 68 and 207 hours after the challenge. This was a significantly lesser mortality in comparison to the present results ($p = 0.0003$).

Our previous finding [2] that splenic artery ligation in animals with induced hypersplenism predispose for development of splenic abscesses, is thus further substantiated by the present results. The reason for the discrepancy between the report of Witte et al. [20] and our results is not fully understood. It might be interesting to study the effect of splenic artery ligation in hypersplenic animals not followed by development of splenic abscesses. An indication that splenic artery ligation not will cause derangement of the defence against encapsulated bacteria was given by Cooney et al. [6] who found normal rats, in which splenic artery ligation had been performed, to be less susceptible to pneumococci than splenectomized rats although more susceptible than normal, non-splenectomized animals. It is not known whether minor abscess formation following splenic artery ligation is more frequent in hypersplenic than in normal rats. However, our data demonstrate that major complications - abscesses - are more frequent in hypersplenic rats after ligation of the splenic artery.

In conclusion, the present report demonstrated that ligation of the splenic artery in hypersplenic rats did not lead to preservation of resistance to pneumococci. Furthermore, this operation caused serious side effect, i.e. abscess formation in the treated animals.

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HYPERSPLENISM - EFFECT ON HAEMOSTASIS

An experimental study in the rat.

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Short title: Haemostasis in hypersplenism.

SUMMARY

Hypersplenism, induced by methylcellulose given to rats intraperitoneally was characterized by splenomegaly, anaemia, leucocytopenia and thrombocytopenia. Hb, Hct, and WBC-count were normalized and platelet count rose to supernormal values by splenectomy.

At a standardized liver resection, the hypersplenic rats had an increased blood loss and prolonged bleeding time in comparison with control rats. Removal of the large spleen normalized these abnormalities.

Hypersplenism also shortened APT-time and impaired ADP and collagen induced platelet aggregation, findings not normalized by splenectomy.

Key words: hypersplenism, splenectomy, bleeding time, blood loss, platelet function.

INTRODUCTION

Hypersplenism, defined as splenomegaly combined with a reduction in one or more cellular elements of blood with hyperplasia of the respective marrow precursors and correction by splenectomy (1) occurs in a variety of disorders.

Portal hypertension, chronic infections, sarcoidosis, thalassemia, storage and infiltrative diseases are well known causes of hypersplenism. Furthermore, this is seen in reactive hyperplasias such as Felty's syndrome and acquired hypogammaglobulinemia, neoplastic and tropical splenomegaly and related disorders (2,3).

Hypersplenism is traditionally treated by splenectomy and indication for this is anemia, repeated infection due to neutropenia, or repeated bleeding because of thrombocytopenia (4,5). Clinical experience indicates that the number of thrombocytes is not enough for the evaluation of haemostasis in the hypersplenic state or the decision for splenectomy. Awareness of increased susceptibility to infectious disease and perhaps also ischemic heart disease has rendered splenectomy controversial (6,7,8,9,10).

These observations led us to evaluate the state of hypersplenism with special reference to the problems around haemostasis. Platelet function, APT-time, blood loss and bleeding time after standardized liver resection were estimated in hypersplenic rats. Hypersplenism was experimentally induced by intraperitoneal injection of methyl-cellulose.

MATERIALS AND METHODS

Male Sprague-Dawley rats weighing 425-460 g were used. The animals were paired at random and ascribed to four groups of sixteen according to the induction of hypersplenism and the performance of splenectomy as follows:

- Group I: Controls receiving intraperitoneal injection of 2 ml NaCl 0.9% for 14 weeks, twice weekly.
- Group II: Controls receiving intraperitoneal injection of 2 ml NaCl 0.9% for 14 weeks, twice weekly; subjected to splenectomy upon completion of treatment; used for the investigation of bleeding tendency 15 days after splenectomy.
- Group III: Hypersplenic. Hypersplenism was induced by intraperitoneal injection of 2 ml methylcellulose 2.5% twice weekly for 14 weeks (Methocel, A4, Dow Chemicals 2160 Stade-Brunnshausen, West Germany).
- Group IV: Hypersplenic, subjected to splenectomy upon completion of the treatment with methylcellulose; used for the investigation of bleeding tendency 15 days after splenectomy.

Under light ether anaesthesia, blood samples (0.6 ml), after transection of 5 mm from the tip of the tail, were obtained for the determination of control values of haemoglobin (Hb), hematocrit (Hct), white blood cell (WBC) counts, and platelet counts (PC). Thereafter half of the animals in each group were subjected to standardized liver resection involving 2.3-2.4% of the total liver weight. Bleeding time and blood loss were determined as earlier described by Bengmark et al (11). The rats were sacrificed by exsanguination after puncture of the aorta immediately after haemostasis was achieved in the liver resected surface. The remaining animals in each group were exsanguinated at the point in time when liver resection was commenced in the paired rat. Blood samples drawn upon exsanguination of liver resected animals were used for the study of haemoglobin, haematocrit, platelet count and APT-time as well as for the study of platelet aggregation.

Haemoglobin (Hb) was determined photometrically according to Kampen and Zijlstra (12). Haematocrit (Hct) was determined in duplicate by the microhaematocrit method and white blood cell (WBC) counts and platelet counts (PC) were determined in a Bürker chamber, using a phasecontrast microscope.

APT-time was counted according to Proctor and Rapaport (13) using commercial standardized reagent with soluble activator (Cephotest, Nyegaard, Denmark).

Platelet aggregation was assayed according to the method of Born and Cross (14), using ADP and collagen as aggregating agents. Platelet rich plasma containing $650-700 \times 10^9$ platelets/lit was utilized as earlier described (11).

Results were statistically evaluated by chi-square test with Yate's correction for small samples.

RESULTS

As demonstrated in Table V A intraperitoneal injection of methylcellulose resulted in ten-fold weight increase of the spleen. There was also a slight increase in liver weight, which persisted 15 days after splenectomy.

Hb, Hct, WBC-count and PC decreased markedly in hypersplenic animals. Fifteen days after splenectomy in methylcellulose-treated rats, Hb and Hct values were normal while WBC-counts and PC increased significantly above the normal (Table VB). Splenectomy in control animals did not affect these values.

Bleeding time and blood loss during liver resection were significantly increased in hypersplenic rats as compared with controls. Two weeks after splenectomy in hypersplenic rats these values were normalized (Table V C). APT-time was markedly decreased in hypersplenic and splenectomized animals before as well as after liver resection (Table VD). ADP and collagen induced platelet aggregation was significantly

decreased in hypersplenic and splenectomized hypersplenic rats as compared with controls (Tables VE, VF). No changes could be registered in control-splenectomized animals.

DISCUSSION

Methylcellulose given intraperitoneally as described by Palmer et al 1953 (15) caused in our rats a disorder very similar to the clinical syndrome called "secondary hypersplenism". Without decreasing weight, the methylcellulose treated animals developed a pronounced splenomegaly. The peripheral blood count changed significantly according to the classical concepts by Doan and Dameshek (2,3). Anaemia and thrombocytopenia in the present model for hypersplenism are believed to be due to a combination of shorter survival and increased pooling of the cells in the spleen (16,17,18,19). The liver is to a much lesser extent involved in the sequestration of blood cells (20,21).

Our experiments showed that "hypersplenism" was associated with a significant increase in bleeding time and blood loss during a standardized liver resection. Splenectomy normalized these values as well as the fall in platelet count. This effect of hypersplenism might be attributed either to an effect on the haemostatic mechanisms or to the presence of haemodynamic changes. Such changes in liver blood flow could possibly explain the increase in bleeding tendency in the present experimental model. Increase in the size of the spleen could be expected to augment the portal flow to the liver; in this case splenectomy would correct the haemodynamic changes and decrease bleeding time and blood loss. Still, the removal of a normal-sized spleen does not have an effect similar to the above, indicating that the increase in liver blood flow cannot be the only cause for the augmentation of the bleeding tendency. Probably, investigation of splanchnic blood flow would provide a more satisfactory answer to this question.

Methylcellulose treatment also resulted in a significant decrease of APT-time, indicating a possible activation of the intrinsic coagulation system. However, the significance and the mechanisms underlying this observation are unclear. Results by Butler et al. (22) showed that APT-time was shortened after splenectomy in hypersplenic patients with Hodgkins disease but these findings were not conclusive. Big molecules, such as dextrans intervene with the intrinsic coagulation system lengthening the APT-time (23), quite contrary to the present noted effect of the methylcellulose molecule. Perhaps a more detailed study of coagulation would reveal whether the shortening of APT-time was due to a true hypercoagulable state or a reactive process following the methylcellulose injection.

The effect of hypersplenism on the number and function of thrombocytes could provide a further explanation for the increase in bleeding tendency. Thrombocytopenia has been a well-recognized symptom of hypersplenism in man and animals. It was also present in our experiment. ADP and collagen-induced platelet aggregation was significantly impaired by hypersplenism. Obviously, not only the number but also the function of thrombocytes was adversely influenced by methylcellulose injections. Removal of a hypertrophic spleen and cessation of methylcellulose treatment resulted in normal values of bleeding time and blood loss and increased values of platelet count, while they did not seem to improve thrombocyte function. Yet, splenectomy in normal animals did not cause any changes in bleeding tendency after liver resection, in haemoglobin, haematocrit, platelet number or platelet aggregation. As demonstrated by Karpatkin (24,25) and Haver and Gear (26) in normal subjects circulating platelets display heterogeneity of size, density and metabolic function, with larger ones showing greater metabolic activity. Possibly, during hypersplenism the most active platelets are sequestered in the spleen and destroyed, while the remaining are not as active haemostatically.

Nagasue and co-workers (27), using Swank's method (28), found increased ADP-induced screen filtration pressure (SFP) even in "normal" splenectomized patients. This disparity might be due to the fact that in Swank's technique blood samples are used immediately after venepuncture for the determination of SFP, without prior standardization of thrombocyte number. It has been demonstrated that platelet aggregate formation, elevating SFP after ADP-stimulation depended on the number of platelets in the sample (29,30). Thus, the successive rise in platelet values, occurring after splenectomy, would be expected to elevate SFP, even in the absence of increased aggregability. Zucker and Mielke (31) and McClure et al. (32) observed that platelet function was not affected significantly in cases of reactive thrombocytosis following splenectomy. McClure et al. (32) mentioned that platelet 5-HT content and uptake were reduced in these subjects and remained abnormal even when PC had returned to normal, thus indicating the presence of a functional defect. However, bearing in mind the differences between human and animals platelets, as well as the fact that several diseases underlying hypersplenism in man might, per se, affect thrombocyte function, one should not compare directly the present experimental results with clinical data.

In clinical practice, however, patients with portal hypertension and hypersplenism often have a troublesome bleeding tendency at operation.

The removal of a large hyperfunctioning spleen mostly has a prompt haemostatic effect combined with a rapid increase in platelet count(5).

In conclusion, we found that methylcellulose induced hypersplenism increased bleeding time and blood loss in liver resected rats. The intrinsic coagulation system seemed accelerated as demonstrated by shortened APT-times, while platelet aggregation was impaired. Correction of hypersplenism by total splenectomy increased the platelet count and normalized bleeding time and blood loss. However,

the shortening of APT-time and impairment of platelet function were not corrected two weeks after splenectomy. The reported results may be relevant for the surgical treatment of patients with hypersplenism.

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TABLE V A

Differences in spleen and liver weight following treatment with intraperitoneal injections of methylcellulose in the rat.

Group	No. of animals	Liver weight, g	Spleen weight, g
I control	16	15.8 $\pm$ 0.5	0.8 $\pm$ 0.01
II control, splenectomized	16	14.5 $\pm$ 0.3	-
III hypersplenic	16	18.0 $\pm$ 0.5	7.8 $\pm$ 0.3 ^s
IV splenectomized	16	17.6 $\pm$ 0.7	-

^s p < 0.01 chi-square test.

Values are presented as mean $\pm$ SEM.

TABLE V B

Hb, Hct, WBC-counts, PC in normal, hypersplenic and splenectomized (15 days later) rats.

Group	No. of animals	Hb g%	HCT %	WBC x 10 ⁹ /l	P x 10 ⁹ /l
I control	16	125.0 ± 1.9	49.7 ± 2.1	14.4 ± 1	987.0 ± 36.1
II control, splenectomized	16	138.7 ± 2.5	48.8 ± 0.5	14.0 ± 2 ^s	939.2 ± 35.0
III hypersplenic	16	99.4 ± 2.1 ^s	38.2 ± 1.8 ^s	8.2 ± 1.5 ^s	580.4 ± 22.8 ^s
IV hypersplenic, splenectomized	16	132.1 ± 3.4	40.0 ± 1.2	16.9 ± 1.2 ^s	1322.7 ± 94.5 ^{s'}

^s p < 0.01 chi-square test.^{s'} p < 0.05.

Values are presented as mean ± SEM.

TABLE V C

Bleeding time and blood loss during liver resection in normal, hypersplenic and splenectomized rats.

Group	No. of animals	Bleeding time sec	Blood loss g
I control	8	223 $\pm$ 11	1.4 $\pm$ 0.3
II control, splenectomized	8	298 $\pm$ 19	1.4 $\pm$ 0.2
III hypersplenic	8	405 $\pm$ 21 ^s	2.5 $\pm$ 0.3 ^s
IV splenectomized	8	264 $\pm$ 21	1.3 $\pm$ 0.2

^s p < 0.01 chi-square test.

Values are presented as mean $\pm$ SEM.

TABLE V D

APT-time before and after liver resection.

Group	No. of animals	APT-time (sec)	
		Before liver resection	After liver resection
I control	8	26 $\pm$ 1	25 $\pm$ 1
II control, splenectomized	8	26 $\pm$ 1	25 $\pm$ 1
III hypersplenic	8	21 $\pm$ 1 ^{s'}	21 $\pm$ 1 ^{s'}
IV splenectomized	8	19 $\pm$ 1 ^s	20 $\pm$ 1 ^{s'}

^s p < 0.01 chi-square test.

^{s'} p < 0.05.

Values are presented as mean $\pm$ SEM.

TABLE V E

ADP-induced platelet aggregation in normal, hypersplenic and splenectomized animals.

Group	No. of animals	ADP (mM)		
		0.1	0.2	1
I control	8	14.9 $\pm$ 0.8	20.7 $\pm$ 1.5	24.1 $\pm$ 1.5
II control, splenectomized	8	14.0 $\pm$ 0.9	19.8 $\pm$ 1.1	23.9 $\pm$ 1.0
III hypersplenic	8	11.5 $\pm$ 0.6 ^{s'}	14.5 $\pm$ 1.0 ^{s'}	20.0 $\pm$ 1.3 ^s
IV splenectomized	8	8.9 $\pm$ 0.9 ^s	11.8 $\pm$ 1.0 ^s	21.9 $\pm$ 1.8 ^{s'}

Values are presented as mean $\pm$ SEM.

Platelet aggregation is expressed as arbitrary units (cm/min) measuring the increase in light transmission (aggregation velocity) in the first 60 sec for each concentration of ADP.

s p < 0.01 chi-square test.

s' p < 0.05.

TABLE V F

Collagen-induced platelet aggregation in normal, hypersplenic and splenectomized rats.

Group	No. of animals	Collagen mg/l	
		10	20
I control	8	17.9 $\pm$ 1.0	22.2 $\pm$ 1.4
II control, splenectomized	8	16.8 $\pm$ 1.1	21.0 $\pm$ 1.2
III hypersplenic	8	14.2 $\pm$ 0.9 ^{s'}	17.4 $\pm$ 1.2 ^{s'}
IV splenectomized	8	9.8 $\pm$ 1.0 ^s	16.6 $\pm$ 0.8 ^s

Values are presented as mean $\pm$ SEM.

Platelet aggregation is expressed as arbitrary units (cm/min) measuring the increase in light transmission (aggregation velocity) in the first 60 sec for each concentration of collagen.

^s $p < 0.01$ chi square test.

^{s'} $p < 0.05$.

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